



University Ss. Cyril and Methodius in Skopje
Faculty of Agricultural Sciences and Food



SYMPOSIUM PROCEEDINGS



*2nd International Symposium for
Agriculture and Food*

*7-9 October 2015,
Ohrid, Republic of Macedonia*

VOLUME I

SKOPJE, 2016

*2nd International Symposium for Agriculture and Food
7-9 October 2015, Ohrid, Republic of Macedonia*

Symposium Proceedings

ISBN 978-9989-845-63-5

**2nd INTERNATIONAL SYMPOSIUM FOR AGRICULTURE AND FOOD
ISAF 2015**

XXXVIII FACULTY-ECONOMY MEETING

V SYMPOSIUM OF VITICULTURE AND WINE PRODUCTION

VIII SYMPOSIUM FOR VEGETABLE AND FLOWER PRODUCTION

**X INTERNATIONAL CONFERENCE OF ASSOCIATION OF AGRICULTURAL
ECONOMISTS OF REPUBLIC OF MACEDONIA**

VI INTERNATIONAL SYMPOSIUM OF LIVESTOCK BREEDING

Organized by
Faculty of Agricultural Sciences and Food of Ss. "Cyril and Methodius" University
in Skopje, Republic of Macedonia
in co-organization with
Institute of Animal Sciences of Ss. "Cyril and Methodius" University in Skopje,
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SYMPOSIUM PROCEEDINGS

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Dear authors,

We are proud to present you with the Proceeding of Papers as an outcome of the Second International Symposium for Agriculture and Food, organized in 2015. This event represents the biggest gathering of the scientific public in the area of agriculture and food in Republic of Macedonia.

Within the framework of the general event several symposiums and conferences took place:

VIIIth Symposium for Vegetable and Flower Production

Vth Symposium for Viticulture and Wine Production

Xth International Conference of the Association of Agricultural Economists of Republic of Macedonia, and

VIth International Symposium for Livestock Breeding.

The Faculty of Agricultural Sciences and Food, University “Ss. Cyril and Methodius” in Skopje, has organized this event for the occasion of its 68th anniversary. It also represents a faculty's commitment and responsibility for conducting and following scientific and research activities as well as for presenting novel results for the relevant stakeholders in the area of agriculture and food.

This event has confirmed the accomplishment of the faculty's vision for being a leading institution in the area of agriculture in Republic of Macedonia, recognized not only in the region but also in Europe and worldwide. The attendance of eminent authors from 21 countries, presenting more than 220 scientific papers, proofs the important role of the faculty for the agricultural and rural development. The papers were presented in 10 parallel sections covering research topics from the area of agriculture, food and environment protection.

The Symposium Proceedings includes 142 scientific papers published in two volumes. The abstracts of all submitted papers (337 in total) were published in Book of Abstracts, prior the organization of the Symposium itself. Part of the submitted papers will be published in the faculty's Journal of Agriculture and Environmental Sciences. Another part of the papers presented at this Symposium will be published in other well-known international journals, as an added value for the scientific importance of this Symposium.

We sincerely hope that with the organization of the Symposium and with the publishing of the Proceeding of Papers we significantly contribute to the science in the area of agriculture and food. We expect that the published papers will be beneficial for the scientists and the experts in their future scientific activities.

Sincerely,

Prof. Dr. Dragi Dimitrievski
President of the Organizing Committee of ISAF 2015
Dean of the Faculty of Agricultural Sciences and Food

CIP - Каталогизација во публикација
Национална и универзитетска библиотека "Св. Климент Охридски", Скопје

631/635(062)
338.43.01(062)

INTERNATIONAL symposium for agriculture and food (2 ; 2015 ; Ohrid)

Symposium proceedings : Електронски извор / 2nd International symposium for agriculture and food, ISAF 2015, 7-9 October 2015, Ohrid, Republic of Macedonia. - Skopje : Faculty of agricultural sciences and food, 2016

Начин на пристап (URL): <http://www.fzhn.ukim.edu.mk/>. - Текст во PDF формат, содржи 2 св. (611 ; 525 стр.). - Наслов преземен од екранот. - Опис на изворот на ден 01.06.2016

ISBN 978-9989-845-63-5 (B. 1)
ISBN 978-9989-845-64-2 (B. 2)

а) Земјоделство - Собири б) Земјоделска економија - Собири
COBISS.MK-ID 101270538



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SECTION 1. ANIMAL BIOTECHNOLOGY

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PANORAMIC APPEARANCE OF THE TURKISH POULTRY INDUSTRYDemirel R.^{1*}, Şentürk Demirel D.¹¹Faculty of Agriculture, Department of Animal Science, Dicle University, Diyarbakır – Turkey

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Abstract

Poultry products are important source of animal protein, especially in low incoming countries such as Turkey. In the Country, most of the animal originated protein is obtained from poultry as egg and white meat. Our population is fastly increasing by the years. Its difficult to meet protein requirements of additional population. Broiler and layer chicks can be easily produced in a short time. At the same time, poult effectively turn the feed to animal protein cheaply. So, it is the easy way to meet protein requirements of increasing population. These advantages make poultry industry getting popular. Timely, traditional poultry breeding in villages turned into an industry in modern conditions with higher yielded animals, good quality feeds etc. From 1980s to 2015, broiler production and meat consumption is reached from 2 kg to 21 kg per capita in Turkey. At the same time, it is placed in the first rank, among animal protein sources. During the period, red meat consumption is decreased from 19 kg to 13 kg per capita. At the same time laying hen production is grown successively, too. Poultry breeders are reach the highest technic knowledge level. There are some constituents of this successfully production such as: good poultry production, using technology, good veterinary services, higher feed quality, hygiene, vaccination etc. In this article, current situation of poultry industry and related sectors in Turkey will be discussed.

Key words: Turkey, poultry industry, broiler, laying hen, mixed feed.

Introduction

Traditional poultry breeding was dominant up to middle of 1980s in Turkey. Especially after the second half of 1980s, the Ministry of Agriculture supported the poultry industry for infrastructure (henhouse, cage, some equipment etc.) between 25 – 40% total cost, depending on developmental situation of the regions.

During the years that increase in internal emigration from rural areas to industrialized cities, traditional food supply become insufficient for the population. So, modern, big scale poultry industry was needed in order to meet food requirements of growing populations in developing cities. Increase in additional peoples lead to raise food requirements of the city population.

Timely, higher yielded modern hybrid lines were used as intensified instead of low yielded domestic combined breeds. Technical knowledge of poultry breeders are better than before. Animal vaccine and medicine, veterinary services became better. As hybrid quality increased, feed quality is also increased in order to meet nutrient requirements of high

yielded animals. Integrated broiler firms are grown fastly. Integrated firms are also essential for farmers who have small scale production. Farmers can obtain chicks, feed, coal, vaccine, medicine, veterinary services etc. without any payment. At the end of growing period, farmers can obtain their profits depending on their success in operation.

Development of Turkish Poultry Industry by Figures

Total produced poultry numbers

Total yearly produced poultry numbers are changed from approximately 264 million to 270 between 2000 and 2014 years as seen in Figure 1.

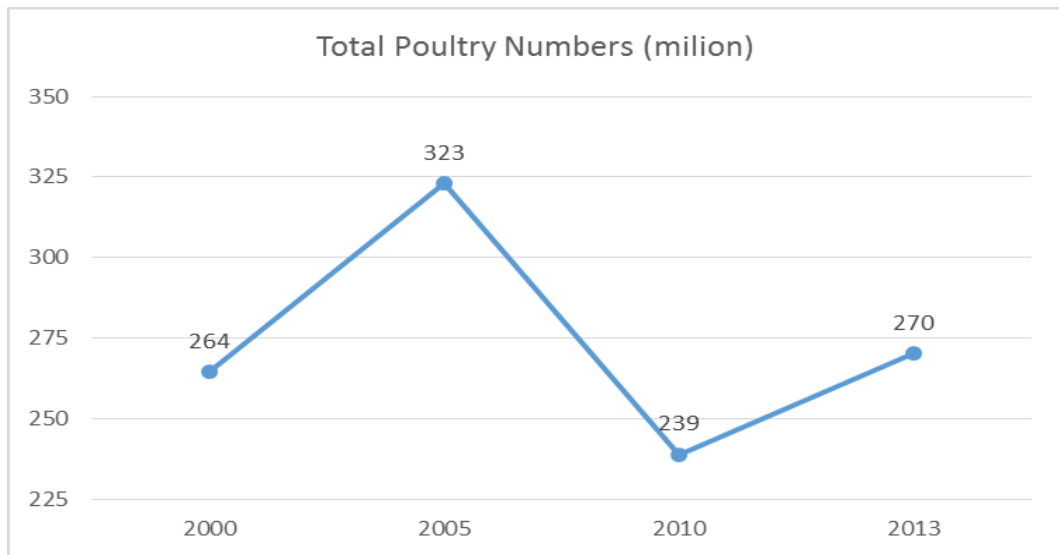


Figure 1. Anonymous, 2015a

Total poultry meat production

Total poultry meat production are changed from approximately 662.000 ton/year to 1.895.000 t/y between 2000 and 2014 years as seen in Figure 2.

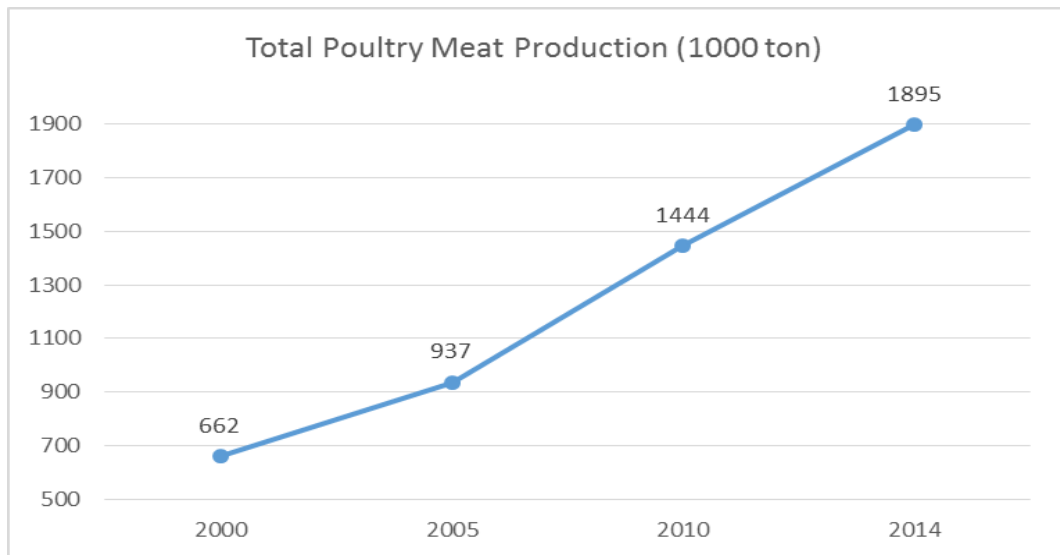


Figure 2. Anonymous, 2015a

Total produced egg production

Total yearly produced egg production (numbers) are changed from approximately 10 billion to 17 billion between 2000 and 2014 years as seen in Figure 3.

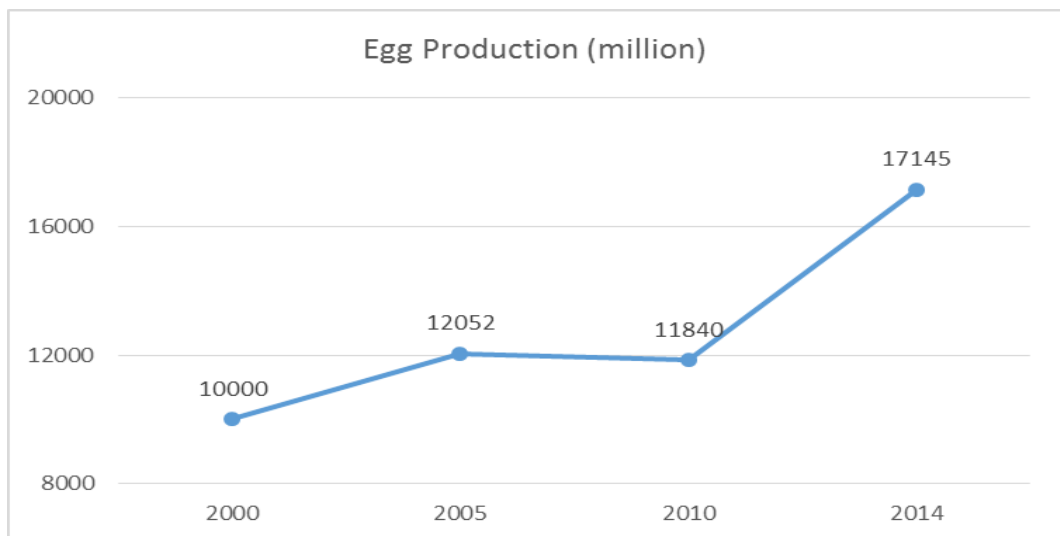


Figure 3. Anonymous, 2015b

Total domestic poultry meat consumption

Total domestic poultry meat consumption is changed from approximately 10 kg to 20,5 kg between 2000 and 2014 years as seen in Figure 4.

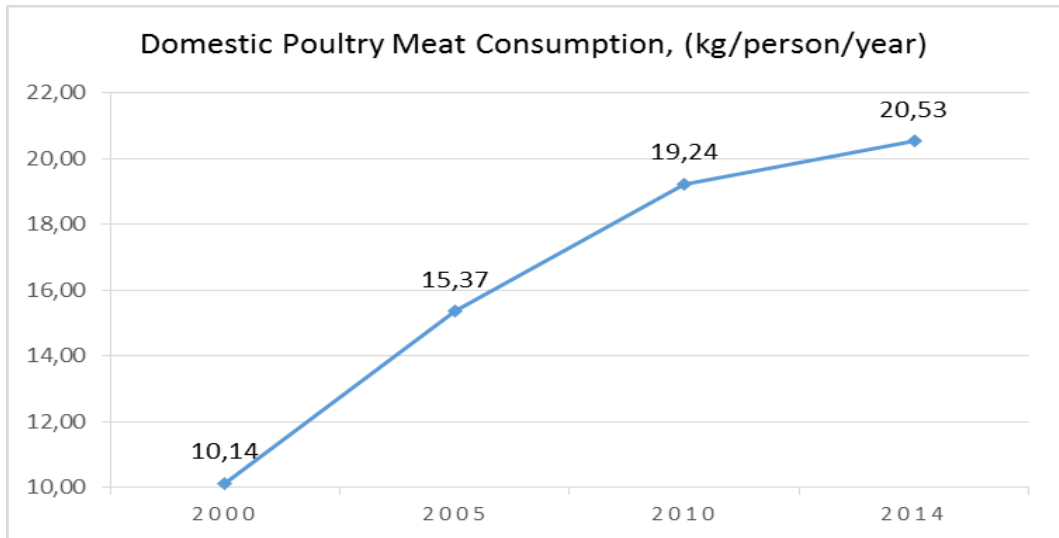


Figure 4. Anonymous, 2015a

Broiler feed production

Broiler feed production is changed from approximately 1 million ton to 3,98 million ton between 2000 and 2014 years as seen in Figure 4.

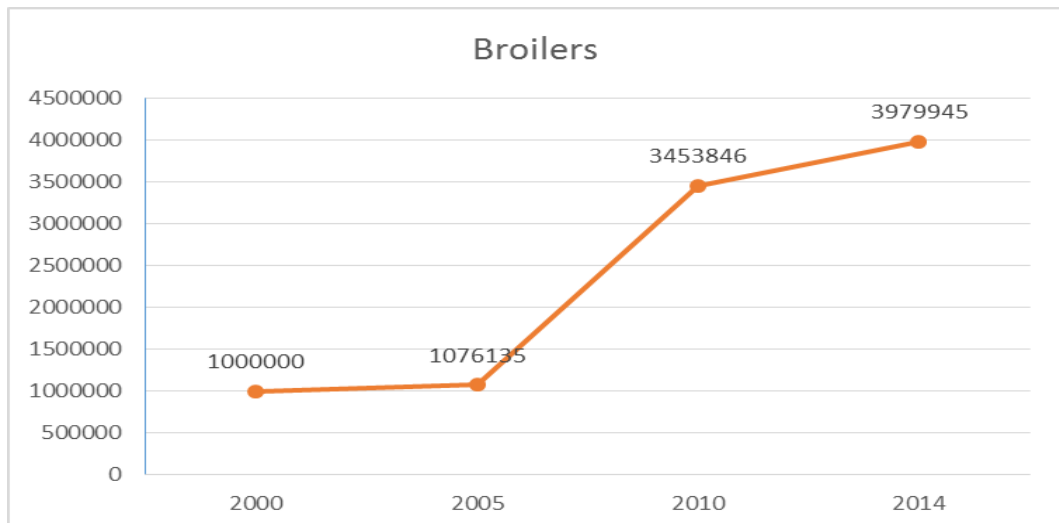


Figure 4. Anonymous, 2015c

Layer feed production

Poultry layer feed production is changed from approximately 80.000 ton to 2,48 million ton between 2000 and 2014 years as seen in Figure 5.

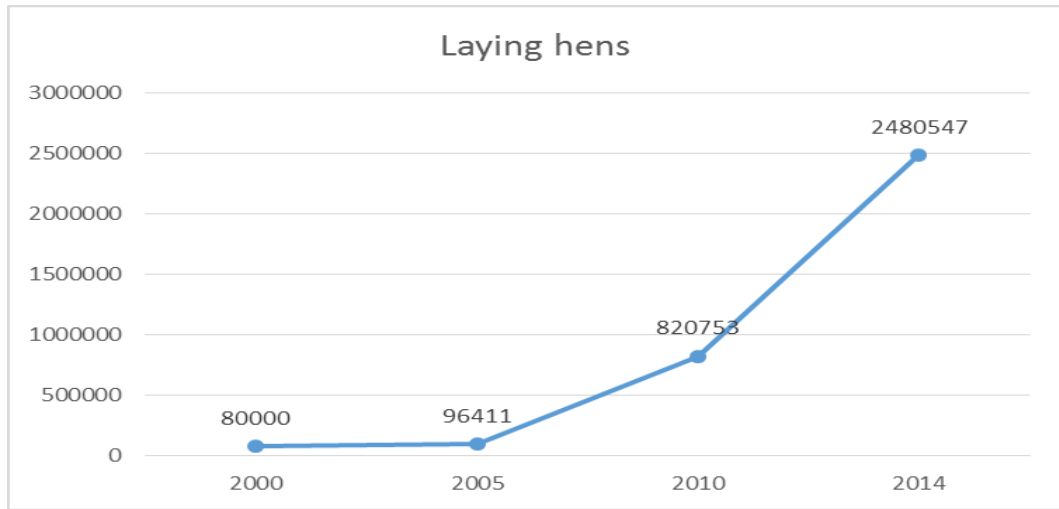


Figure 5. Anonymous, 2015c

Poultry meat export

Total poultry meat export has changed from approximately 24,417 ton to 395,694 ton between 2000 and 2014 years as seen in Figure 6.

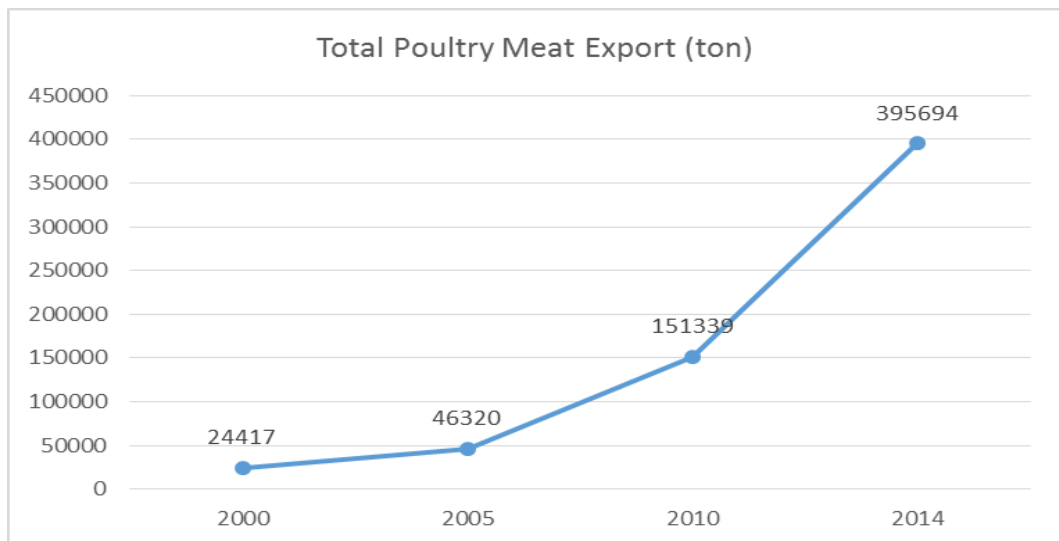


Figure 6. Anonymous, 2015d

Conclusions

Turkish poultry industry is growing fast, however consumption of domestic poultry products is growing slowly. So, some of Turkish poultry products are sent to export. Poultry feed constituents are commonly imported (soybean, fat and oils, additives etc.) and these figures are getting high year by year. Our production figures (feeding period, mortality, product quality, FCR, etc) are competitive with developed countries, but our farms are low

scale and profitability levels are lower. At the same time, our production is considered as halal production. High quality and halal production is getting more popular for international markets.

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BROILER PRODUCTION IN MACEDONIA

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Abstract

Macedonian broiler industry is underdeveloped therefore majority (over 90%) of the poultry meat on the market is imported frozen poultry meat. Initial steps for development of broiler sector are focusing on development of chilled poultry meat market. Market analyze revealed that possibilities for developing the sector exists and domestic market trends follows the international poultry market trends. Projections based on these trends, following demands and supply suggest substantial market size growth and shift leading to increased market share of chilled poultry meat and meat products toward frozen one in next ten to fifteen years. Based on the information on domestic production as well as on the import data it can be concluded that activities are needed in all aspects of the supply chain starting from broiler raising and slaughtering to processing up to sales and distribution of chilled and frozen poultry meat. Once the basic elements of well organized chilled broiler meat supply is established there will be further opportunities open for the other value added products such as chicken sausages, chicken burgers and nuggets (for the fast food industry).

Key words: poultry meat, market, trends, projections.

Introduction

Macedonian poultry industry satisfies the domestic egg market needs (MAFWE, 2007), but at the same time Macedonia is importer of a poultry meat. The level of meat production of our poultry industry is presented in Table 1 (MAFWE, 2007; FAOSTAT, 2012). In addition, the half of the mentioned production of poultry meat (broiler meat) is attributed to a spent layers meat (chicken meat) (MAFWE, 2007).

Table 1. Quantity of poultry (chicken) meat production

Year	Total raw meat produced, tons
2010	3200
2009	3319
2008	3012
2007	3524
2006	3715
2005	3 809
2004	3 189
2003	4 116
2002	3 992
2001	4 702

The total poultry meat consumption in Macedonia is 23 000-30 000 tons per year, or 11.5-14.5 kg/per capita (according National Statistic Office data, the 2005 annual consumption of poultry meat per capita was 11.0 kg suggesting a market needs of 22 000 t/year, while the Veterinary Directorate reports import of 24. 093 t, which is close to the poultry meat imports of FAO (Table 2).

Table 2. Production and import of poultry meat in the R. Macedonia

(1000 tons)	Macedonia							
FAOSTAT FAO Statistics Division September 2012	2003	2004	2005	2006	2007	2008	2009	2010
Production	4.12	3.19	3.81	3.71	3.52	3.01	3.32	3.20
Import	20.74	25.74	21.49	19.69	30.84	25.41	28.78	25.99

The EU information (EUROSTAT) about the chicken meat consumption in European Union and projections for the poultry meat market movement and chicken meat consumption per capita suggest that Macedonia, considering the poultry meat consumption, is behind states members of EU (Table 3)

Table 3. Consumption data for poultry meat in R. Macedonia and EU, 2003-2012 (kg per capita) (FAOSTAT)

	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
EU-25	22.9	22.9	23.6	23.6	23.8	23.9	23.2	23.1	22.6	22.8
R. Macedonia	13.5	15.8	13.9	13.2	19.40	16.00	17.30	17.00	16.90	17.10

Materials and methods

Questionnaire research survey on consumer preferences of poultry meat focused on chilled broiler meat marketing development idea, including 1000 examinees, revealed that the most of the questioned consumers purchased poultry meat more than three times a month. Only 20.8% buy chilled poultry meat regularly, beside the fact that 83.5% recognize chilled and frozen poultry meat as different product. Only 45.4% of the questioned consumers (about a half) preferred to buy the most valuable and highly priced meat parts of first class meat (breast, drumstick and thighs). The majority of consumers 89.2% accept higher prices for better quality articulated to price/quality relations. Quality is dominant factor and a crucial moment in buying decision 67%, followed by health 59.4%, but due to

its relativity price is still a factor that affects the decision due to the low buying power of the consumers.

If the trend of the Macedonian market follows the consumption and quality demand of the new EU member states, (where fresh (chilled) poultry meat will supersede the most of the market), fresh (chilled) poultry meat will increase the domestic market needs, following the needs of our consumers for fresh chilled chicken meat (whole chicken, breast, drumstick and thighs). This trend is occurring beside the higher price of products of fresh (chilled) poultry meat. The price for fresh (chilled) poultry is 115-169 denar/kg i.e. 1.9 EUR-2.8 EUR; the price for frozen chicken meat is 90-120 denar/kg i.e. 1.46-2.1 EUR.

Based on the data from 2005, predictions can be made on the growth of the poultry meat market in the next years in order to know the situation after 5, 10 and 20 years. Considering the basic assumptions from the Table 4, than the given figure will present the market growth potentials and a part of the domestic production and potentials for increasing the broiler industry in the next 20 years.

Table 4. Poultry sales past and predicted future

	2005: data	2010: data	2015: forecast
Number of inhabitants	2.0 million people	2,1 million people	2.2 million people
Total poultry meat consumption	25.300 tonnes	29.190 tonnes	33.000 tonnes
Poultry meat consumption per capita	12.66 kg/capita	13.90 kg/capita	15.00 kg/capita
Total poultry meat supply:			
- Meat from a spent layers	1.810	1.000	1.000
- Fresh broiler meat	2.000	2.200	7.000
- Import	21.490	25.990	25.000

Conclusions

Considering these predictions and projections according to development state policies designed in national and IPARD program, followed by the program for financial support in the agriculture (30 MKD i.e. 0.47 Euro/broiler for 2011), it is probable to expect growth and development of broiler industry in the next period. If current level of production is about 2200 tones/year covering the market demands with approximately 7%, as soon as production starts to increase, the level of about 25% should be reached within 5 years and about 30% within 10 years.

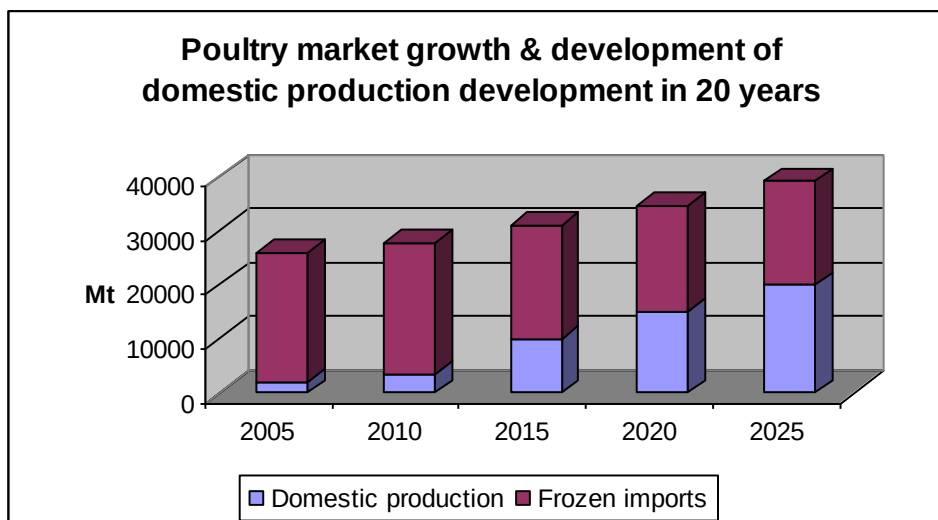


Figure 1. Predicted market growth and development of broiler production

Following expected trends presented in Table 4 and Figure 1, it could be expected that the part of the frozen poultry meat in total offer will decrease (from 87% to 63% by 2015 and 46.7% by 2025) as the fresh chilled poultry meat from domestic production increase its part on the market supply (from 7% to 30% and 48.4%, respectively).

Generally, according to the analysis presented above, there is a conclusion that there are possibilities for broiler production, focusing on the domestic market for fresh (chilled) poultry meat.

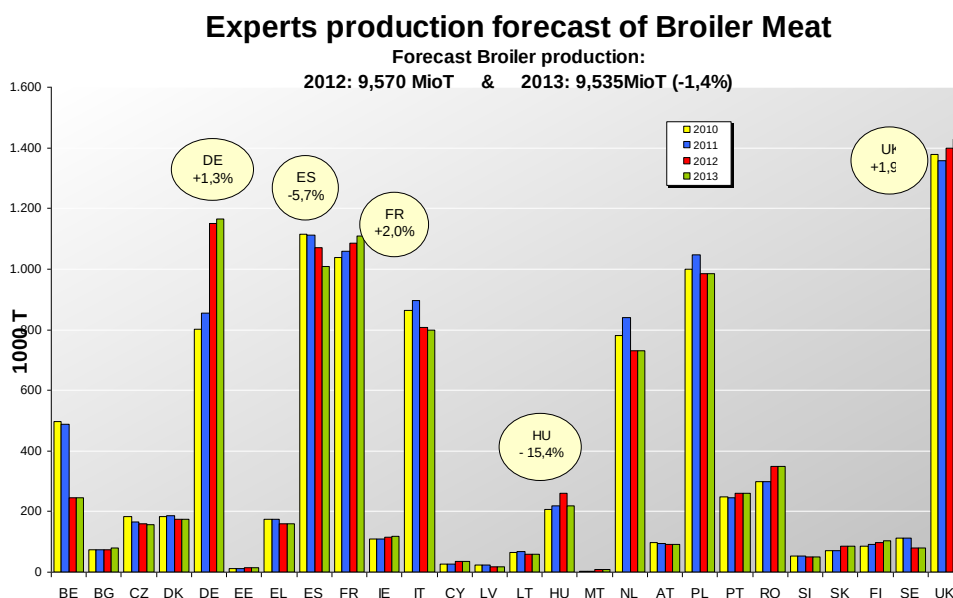


Figure 2. Predicted production forecast of broiler production in EU in 2013 (CIRCA).

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PHENOTYPIC CHARACTERIZATION OF BUSHA CATTLE IN THE R. OF MACEDONIA

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Abstract

Busha cattle are indigenous breed in many Balkan countries. Because of the economic, cultural and scientific reasons it is very important to protect biological diversity of autochthonous breeds like busha cattle. The aim of the research was to estimate the main productive, reproductive and exterior traits in several strains of adult busha cattle in the R. of Macedonia. The average daily milk production in grey strain of busha cows was 3,8 kg, and in brown strain was 4,1 kg, with 3,89 i.e. 4,01% of fats, 3,57% i.e. 3,62% proteins and dry unfatted matters 9,49% i.e. 9,62% in milk from brown i.e. brown strain of busha cows. According to the measurement of some morphological traits of adult busha cows, in grew strain the wither height was 107 cm, in black strain 105, and in brown strain also 105 cm, with the similar values for the traits back height and rump height in cows. The length of head was 38 cm i.e. 37 in grew i.e. black and brown strain, and length of horns 16 i.e. 15 cm in grew and black i.e. brown strain of cows. The average body mass of newborn calves was 15 kg i.e. 14 kg in grew and brown i.e. black strain. The average body weight at first mating was 125 kg in male and 150 kg of female busha cattle, and the average age at first calving was 28 months. The research results have shown small variation in their productive, reproductive and morphological traits between different strains of busha.

Key words: cattle, Busha, domestic breed, phenotypic traits.

Introduction

Cattle are a main source of milk and beef products in Macedonia including various breeds, strains and crosses. This industry consists of three sectors: small-scale farmers (around 90%) keeping 1-3 cow and mainly producing for home consumption; medium-scale farmers (5%) keeping 10-15 cows with annual production of 4000-5000 kg milk/head and specialized commercial farms (around 5%) with more than 50 heads that produce annually over 7.000 kg milk intended solely for the market, or beef production with more than 400 kg body mass per year per head.

In the mountain rural regions of Macedonia the dominant type of cattle are the crosses of the Busha breed. This breed of Busha cattle, also known as Illyrian cattle, is

autochthonous breed of the Balkan Peninsula. It has been bred for centuries in this area and belongs to a group of primitive short horned cattle (*Bos brachyceros Europaeus*).

Busha cattle is an indigenous, actually trans-boundary breed, which is in high danger of extinction. It used to be dominant and most important breed in almost all Balkan countries until 50s and 60s of the XX century but today in lowland areas with intensive farming they are already replaced with more productive and specialized cattle breeds. In Macedonia this breed was officially classified as triple purpose breed (for meat, milk and work) but considering its low productive capabilities it is more similar to some primitive working breeds. Today these cattle are no longer used for work but because of absence of systematic cattle improvement program these animals have retained their poor beef and dairy production capability. It could be said that the Busha's genome is very elastic since this breed in unfavorable conditions easily achieves better milk production and bigger body weight. Having in mind that this breed is well adapted to the very harsh feeding and housing conditions that exist in the rural areas of the Macedonian mountains and is resistant to diseases, it is still the most significant beef and sometimes milk resource for these areas where the more productive cattle breeds cannot thrive successfully.

In the past several decades, as a result of uncontrolled crossing of this cattle with some more productive breeds, the number of purebred Busha animals is permanently being reduced which imposes an urgent need for setting up *in situ* and *ex situ* conservation program for this breed. Because of the economic, cultural and scientific reasons it is very important to protect biological diversity of autochthonous breeds like Busha.

Busha strains in the R. of Macedonia

There are two main classifications of Busha strains in the R. of Macedonia:

a) Classification of strains according to locality:

- Povardarie strain,
- Polog strain,
- Orgazden strain,
- Prespa (Ohrid) strain,
- Mariovo strain, etc.

b) Classification of strains according to color:

- Black strain,
- Brown strain,
- Red strain,
- Gray strain, and
- Tiger strain.

In our country the following varieties on the basis of their coat color can be found: black Busha which is reared in Debar, Tetovo and Gostivar region (Polog strain), red Busha (Metohija strain), grey Busha (Povarie strain and Prespa or Ohrid strain), brown strain (Orgazden strain) and sometimes the so called “tiger” strain.

Actual situation of cattle breeding in the R. of Macedonia

According to the official statistical data there are totally 214.000 cattle in the R. of Macedonia (FAO, 2014), from which 12.064 heads of Busha cattle or 5,6% (Agency of Veterinary and Food-AFV, 2014), but according to reality, there are approximately 1000-2000 heads of Busha cattle in our state.

State policy and legislative for conservation of Busha cattle

In 2008 a new Law of animal production is in function in the R. of Macedonia, where several articles are regulating the biodiversity and species of autochthonous breeds of cattle in Macedonia. From cattle, the only autochthonous breed is Busha cattle.

In 2010 a Livestock Common Breeding Program was established from the Ministry of Agriculture of RM, for the period from 2010 to 2020, where the main stress from cattle breeds was given to Busha cattle. According this program, a Separate Breeding

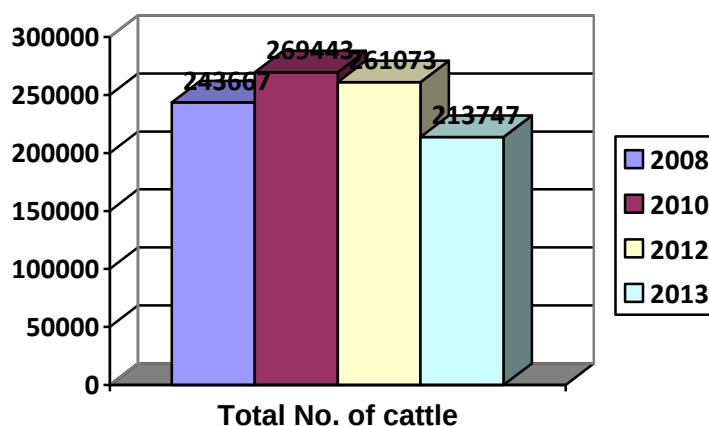
Programs for each breed have to be done, for the duration of 5-10 years. In 2011 was done the Program for Livestock Biodiversity by the Ministry of Agriculture of RM, for the period 2011-2017, with the main activities:

- Inventarization,
- characterization, monitoring of the trends and risks
- Phenotype analyses,
- Production traits,
- Reproduction traits,
- DNA characterization, etc.

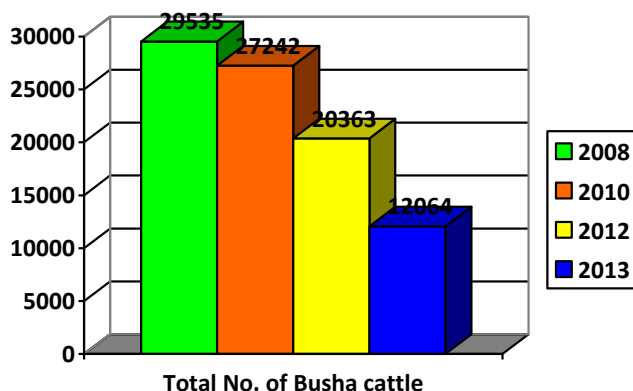
The overall objective of the National Breeding Program for Busha cattle is to implement the national conservation plan and sustainable use of Busha cattle.

Table 1. Cattle breed distribution according the official data in the R. of Macedonia (AVF, 2014)

Breed	2008		2010		2012		2013	
	No. of heads	In %	No. of heads	In %	No. of heads	In %	No. of heads	In %
Busha cattle	29535	12,1	27242	7,8	20363	10,1	12064	5,64
Crosses of Busha and other breeds	89707	36,8	104961	43,6	113720	39	98958	46,3
Total No. of cattle	243667	100	269443	100	261073	100	213747	100



Graph. 1 Number of cattle in RM (AFV, 2014)



Graph. 2 Total No. of Busha cattle in RM (AFV, 2014)

Material and methods

Several herds of busha cattle were investigated for the main productive, morphological and reproductive traits, mainly from south-eastern, south and south-western part of the R. of Macedonia, from hill-mountain area. All of them are reared semi-nomadic, i.e. during winter period from the end of October till the middle of April in the mountain village areas, and on the warm part of the year they are migrated on the mountain pastures according to the water sources and pasture yield. The controlled Busha cattle are mainly aimed for beef production, but some of them are milking by hand only for the purposes for farmers, not for selling. From the dairy production traits, the main emphasis was given to the milk yield per day and milk content (percentage of fats, proteins, solids non-fat matters and dry matters in milk), from the beef production traits to the average daily gain and body mass on 6 and 12 months of age and meat percentage from the carcass. The main morphological traits were measured with Lithin's rod and measure tape, only on adult Busha cattle. Also, several main reproductive traits were measured, with the emphasis on the age on first mating and delivering, duration of open days and calving period, as well as on birth weight and body weight.

From 2012 the 5 main bigger Busha farms were under the control of their productive and reproductive characteristics, from different regions of Macedonia: Herd 1 – Prilep region: 450 cattle, Herd 2 – Ograzden region: 120 cattle, Herd 3 – Ohrid region: 44 cattle, Herd 4 – Rozden region: 172 cattle, and Herd 5 - Belcista region: 94 cattle. In 2013 there were under the control 11 Busha herds, and in 2014 totally 38 Busha herds with approximately 800 heads of adult Busha cattle in the R. of Macedonia.

Results and discussion

Morphological characteristics

When compared to the higher productive European dairy and beef breeds, Busha animals are of much smaller size. Carried by a short neck their head is small and light with short horns pointing upward and forward.

Table 2. Morphological traits of Busha cattle in Macedonia

Trait	Grew strain	Brown strain	Black strain	Total x
No. of cows	11	15	4	30
Wither height	107 (104-111)	105 (99-113)	105 (103-108)	106
Back height	107 (103-111)	105 (101-114)	105 (103-109)	107
Rump height	109 (103-114)	108 (102-117)	107 (104-115)	108
Length of head	38 (33-42)	37 (31-40)	37 (35-41)	37
Length of horns	16 (14-21)	16 (12-19)	15 (14-18)	16
Born weight	15 (12-18)	14 (11-17)	15 (12-17)	15

The fully grown Busha animal is 90-115 cm tall at the withers. The length of the body is 116-132 cm or 104-118% of the withers height. There are great variations in the length of the body which is a result of the extensive way of rearing, lack of any breeding program and the poor zootechnical measures. Some of these animals have 1-3% higher pins than withers and some are with horizontal back line. They have narrow chest whose width is approximately 27% and depth 50-55% of the withers height (Ilkovski, 1994, Bunevski, 1994, Trajkovski and Bunevski, 2006). The fore limbs are straight but the hind limbs are usually hocked in. The rear part is narrow and the legs are sturdy with strong joints and ligaments. The hooves are hard and strong which enables these animals to move easily on steep and rocky terrains. The udder is small and shallow but with regular shape. Those animals that are better fed usually have bigger and deeper udders with more glandular tissue. The body weight of the fully grown animals is 150-300 kg but with better nutrition the cows can grow up to 280-320 kg and the bulls up to 430 kg (Trajkovski and Bunevski, 2006). The small body weight of these animals is not completely genetically dependent but also is a result of generations of underfeeding and neglecting. The skin is elastic and hard. Those animals that are reared well have short and shiny coat hair while in those that are neglected the coat is long and dense. Those that are raised on high mountain pastures always have longer hair. They usually have a stripe over the back and the muzzle, the horns and the hooves are darkly pigmented. Similar morphological results were obtained also for the other strains of busha in the neighboring countries from Croatia to Turkey (Kume K. et. Al., 2013).

The weight of adult males is between 250 and 300 kg, females between 150-250 kg. Their average wither height is 105-115 cm (Bunevski, 1994, Kume K. et. all, 2013). The head is characterized with short and curved horns pointing upward and forward and light pigmentation around the eyes and the muzzle. It is very robust and resistant with very modest feeding and housing demands. They are well adapted to the very harsh feeding and housing conditions, resistant to diseases and have long production life - 10 and more lactations. It could be said that the Busha's genome is very elastic, since this breed in more favorable conditions easily achieves bigger body weight and much better production.

Productive characteristics

In general these animals have low production which is in accordance with the poor body conformation. On the basis of the production capabilities this breed can be classified as triple purpose breed (for milk, meat and work). The low milk production is a result of the poor nutrition and the underdeveloped udders. Because of the small body weight it is a poor working animal and because of its poor body conformation and underdeveloped meat (beef) portions it is a weak fattening animal. The milk production of this breed is

from 700 to 1500 kg with 3,7-4,0% milk fat but there are also animals with higher milk production with around 2400 kg or with 4,8% milk fat. The lactation period lasts around 240 day. This low production can not compete with the specialized dairy breeds and the European countries with highly developed cattle breeding practices abandoned or rearing this breed long time ago. The milk content is shown in the following table.

Table 3. Milk components from Busha cows

n	Strain	Fats %	Dry Unfatted matters %	Protein %	Kg milk/day
34	Grey strain	3,89 (3,65-4,45)	9,49	3,57	3,8 (2,8-5,3)
28	Brown strain	4,04 (3,79-4,61)	9,62	3,62	4,1 (3,1-6,2)

The fattening capabilities of Busha cattle are relatively poor. The birth weight of the calves is 15 - 22 kg and the daily weight gain around 500 gr. The meat is also of poor quality. The fattening capabilities are low because of the under nutrition and delayed maturity. Well fed male castrated young bulls have dressing percentage of around 47-55%. The fattened castrated males reach weight of 280-400 kg. The meat is of low quality. The health and the conformation of these animals is generally satisfactory having in mind that in such a bad conditions no other specialized cattle breed can survive.

Reproductive characteristics

Busha breed is classified as late-maturity breed because it reaches puberty at 13-15 months of age, breeding maturity when 2-2,5 years old and full growth at 4 years of age. The first calving of heifers is usually when they are 28 months of age (tab. 4).

Table 4. Reproductive traits of Busha cattle in Macedonia (n =33 male and 54 female adult cattle)

Trait	Male	Female
Age at first calving	/	28 months (19-39)
Weight at first mating	125 kg (95-155)	150 kg (125-200)
Age at first mating	After 12 months	28 months
Fertility (%)	/	55%
Birth weight (kg)	15	14
Body weight (kg)	275 (245-390)	230 (180-360)
Age at 12 months of age	125 kg (95-155)	150 kg (90-182)

Under good raising and feeding conditions the fertility of this breed is generally good but in poor conditions when underfed they easily develop anovulation due to hunger. The bulls reach breeding maturity at 2,5 to 3 years of age while the cows after calving usually have short period of days open from 4 to 6 weeks and can give birth 10-13 times in their life (Ilkovski et al, 1994, Bunevski et al, 2004). Duration of days-open period has a big variation in our population, from 20 days after delivering till 100 days, as well as the duration of calving period from 300 to 400 days. These cattle show high disease resistance and adaptation to deficient nutritional and other environmental conditions.

Conclusions

The aim of the research was to show the main productive, reproductive and exterior traits in several strains of adult Busha cattle in the R. of Macedonia. The average daily milk production in grey strain of Busha cows was 3,8 kg, and in brown strain was 4,1 kg, with 3,89 i.e. 4,01% of fats, 3,57% i.e. 3,62% proteins and dry unfatted matters 9,49% i.e. 9,62% in milk from brown i.e. brown strain of Busha cows. According to the measurement of some morphological traits of adult Busha cows, in grew strain the wither height was 107 cm, in black strain 105, and in brown strain also 105 cm, with the similar values for the traits back height and rump height in cows. The length of head was 38 cm i.e. 37 in grew i.e. black and brown strain, and length of horns 16 i.e. 15 cm in grew and black i.e. brown strain of cows. The average body mass of newborn calves was 15 kg i.e. 14 kg in grew and brown i.e. black strain. The average body weight at first mating was 125 kg in male and 150 kg of female Busha cattle, and the average age at first calving was 28 months. The variation in their productive, reproductive and morphological traits between different strains of Busha is good for choosing superior bulls and cows for genetic improvement of this breed.

In the past several decades, as a result of uncontrolled crossing of this cattle with some more productive breeds, the number of purebred Busha animals is permanently being reduced which imposes an urgent need for setting up *in situ* and *ex situ* conservation program for this breed. Because of the economic, cultural and scientific reasons, it is very important to protect biological diversity of autochthonous breeds like Busha cattle.

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GROWTH AND DEVELOPMENT OF SHEEP FROM SYNTHETIC POPULATION BULGARIAN MILK

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Abstract

The goal of this study is to investigate the growth and development of sheep from breed Synthetic Population Bulgarian Milk (SPBM) at different ages and to define genetic determination of the trait by age categories.. The animals are from the herd of the Agricultural Institute – Shumen. The survey covers 851 ewes born during 2004 – 2008. Body weight of nine-month female lambs from this breed is 91.06% of weight of the 1.5 year female animals and 65.65% of that on 2.5 years (70.97 kg). The highest values of the coefficient of variation ($C=21.309$) has live weight at 100 days of age, while the next age periods shows a downward trend, reaching 8.136% at 2.5 years. The father' line has a fairly high impact on the live weight at 9 months ($P\leq 0.001$) and less at 1.5 and 2.5 years of age ($P\leq 0.01$). Year of birth has a fairly high impact on the trait live weight of all ages ($P\leq 0.001$). The coefficient of heritability (h^2) of the live weight categories ranging from 0.094 to 0.340.

Key words: sheep, Synthetic Population Bulgarian Milk breed, growth and development, breeding line.

Introduction

Synthetic Population Bulgarian Milk (SPBM) sheep has been recognized as a breed in 2005 and is now the most widespread in Bulgaria. It was created by applying the hybridization of different maternal basis using rams of breeds East-Friesian, Pleven Blackhead, Stara Zagora and Awassi. A large part of the studies performed until now, relate to the parameters defining the productivity in the various stages of the population creation (Lazarov et al., 2002; Nedelchev et al., 2003; Raicheva et al., 2003; Stancheva, 2003; Boikovski et al., 2005b) and to establish the effect of certain factors (as order of lactation, year of birth, linear differentiation, age of mating, type of udder and method of calculation) on the variability of phenotypic parameters of milk yield traits and biological fertility (Boikovski et al., 2003a, 2003b, 2005a, 2006, Stancheva et al., 2006, 2014; Ivanova and Raicheva, 2008; Hinkovski et al., 2008; Raicheva and Ivanova, 2010, 2011a, 2011b).

Scientific approach to the application of the methods for selection and productive traits monitoring in SPBM sheep herds of Agricultural Academy resulted in identifying the productive parameters, characterizing them as the best for growing rams and consolidated

growth and high body weight (Raicheva et al., 2010). This is one of the prerequisites for getting a high milk yield. In elite herds there are genealogical (genetic) structures having an extremely high body weight (Stancheva, 2003). It is planned in future selection to be carried out optimization of live weight in order to maximize milk yield traits. The importance of the trait live weight in sheep breeding is determined by the fact that the high energy growth at a young age and realized other productivity determine the effectiveness of this sub-sector of livestock. Together with the other traits typical for different productive directions, body weight defines the parameters of the standards of individual sheep breeding lines. Bodyweight is selectable trait that is controlled in sheep of all productive directions and is known that is influenced by genetic and non-genetic factors (Mandal et al., 2003; Behzadi et al., 2007; Dass et al., 2008). The dynamics of phenotypic realization during the selection process represents undeniable interest.

The goal of this study is to investigate the growth and development of sheep from breed Synthetic Population Bulgarian Milk (SPBM) of Agricultural Institute - Shumen at different ages and to define genetic determination of the trait by age categories.

Materials and methods

The survey covers 851 number ewes from Synthetic Population Bulgarian Milk, born during 2004 - 2008 in the flock of Agricultural Institute - Shumen. Based on available information of stud-book are set pure-breeding lines in the herd (27, 522, 913, 942, 976, 1254, 3156, 6988, 8265 and 88550) and experimental group with Chios blood (51). The trait live weight was studied on different age. Live weight was measured individually at weaning (on 100 days), 9 months old, 1.5, 2.5 years monitored and recorded by standard methods and guidelines laid down in the Instructions for performance monitoring and rating of sheep SPBM (2003, 2013). It was calculated variance estimations which influence some sources of variation on weight development. F-criteria of reliability of each of them were established. It is found genotypic effects (h^2 - heritability) of live weight at different ages (Harvey, 1990).

Results and discussion

It was found that during the experimental period, the live-weight of 9 months (46.59kg) measured in female SPBM hoggets represents 65.65% of that of the animals of 2.5 years (70.97 kg) and 91.06% - of 1.5 years (64.63kg) (Table 1).

The variation in body weight in different age periods is distinguished. The highest value of the variation coefficient ($C = 21.31\%$) was observed in live weight at weaning, followed by those for subsequent age groups in descending order, reaching 8.14% at 2.5 years. This is a logical consequence of the applied practice of pure-line breeding in the study herd. The main selection trait in SPBM sheep is lactation and on this reason allowed for breeding in some cases lambs with less body weight, which in later ages compensate the weight development. The indicator value for accuracy of the mean (E) are low - from 0.33% to 0.73%, and show that the results are representative of the general population.

SECTION 1. ANIMAL BIOTECHNOLOGY

Table 1. Overall average and coefficient of variation of the live weight in SPBM sheep

Live-weight	n		S	C %	E
at weaning	851	28,71	6,12	21,31	0,73
on 9 months	787	46,59	5,24	11,44	0,41
on 1.5 years	702	64,63	6,57	10,17	0,38
on 2,5 years	592	70,97	5,77	8,136	0,33

Table 2. Weight growth of SPBM sheep depending on the breeding line

№	line	Live-weight											
		at weaning			on 9 months			on 1.5 years			on 2.5 years		
		n	x	C	n	x	C	n	x	C	n	x	C
1	27	20	28,10	14,96	17	47,82	10,36	17	64,12	8,45	12	69,33	4,70
2	51	20	25,65	13,28	20	47,40	12,42	18	70,22	7,45	16	74,44	5,48
3	522	107	28,72	21,85	98	47,54	10,42	88	64,67	8,49	71	70,25	6,62
4	913	64	30,14	18,89	56	46,18	9,65	51	65,55	11,11	44	71,18	9,08
5	942	97	30,53	27,76	94	43,42	11,73	81	63,25	11,49	75	69,21	8,38
6	976	39	30,05	23,76	36	45,67	14,07	28	62,86	11,90	22	68,96	8,06
7	1254	99	27,80	20,29	87	46,22	9,79	71	63,85	11,19	54	72,54	8,18
8	3156	222	28,19	19,31	206	47,21	11,08	189	64,51	9,72	170	70,99	7,77
9	6988	57	27,63	18,84	52	46,81	14,30	49	64,51	8,32	39	71,36	9,71
10	8265	26	26,23	22,42	26	44,73	9,00	25	64,80	9,22	23	72,35	7,59
11	88550	83	30,15	18,80	79	47,92	9,61	71	64,44	10,25	52	70,71	9,19
	total	834	28,75	21,40	771	46,51	11,37	688	64,47	10,09	578	70,87	8,14
Significance of differences at:													
$P \leq 0.001$	2-3,4,5,11			5 - 3,4,7,8,9,11; 11-10			2 - 3,5,6,7,8,9,11			2 - 3			
$P \leq 0.01$	2-6; 7-5,11; 9-5,11; 10 - 4,5,11			5 - 1,2; 8 - 10;			2 - 1,4,10			2 - 1,6,11			
$P \leq 0.05$	2-7,1; 4-8; 7-4; 9-4; 10-6			11 - 7,4; 1 - 10;						2 - 4,9; 7 - 1,3,6; 10 - 1,6;			

The analysis of the weight development depending on the breeding line (Table 2) shows that animals from line 51 (with Chios blood) at weaning reach the lowest live weight - 25.65kg compared with those of pure-bred lines of SPBM. This we believe is due to genetic (type of birth, maternal effect) and environmental factors and is consistent with those reported by Fernandes et al. (2001); Nedelchev et al. (2005); Kuchtík and Dobeš (2006); Petrović et al. (2012) and Taskin et al. (2012) results in similar kind of studies.

With the highest live weight were lambs of 942 line (30.53kg), followed by its coeval of breeding lines 88550 (30.15kg), 913 (30.14kg) and 976 (30.05kg). Our results for the animals of SPBM and those with Chios blood are higher than those reported by Djorbineva et al. (2008). The lower values of the live weight at weaning of SPBM animals reported by Nedelchev et al. (2003); Hinkovski et al. (2008) and Iliev (2013) are probably due to the different weaning age and the environmental conditions. The differences between the individual lines have been demonstrated in different degrees of significance ($P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$). The inside-linear variability of the live weight at weaning shows medium to very high values ($C=13.28$ to $C=27.76\%$).

On nine months between the lines there is some change in weight development. Animals from the breeding line 942 of SPBM that at weaning were recorded the highest live-weight now presented the lowest among all other - 43.41kg ($P \leq 0.01$, $P \leq 0.001$), with

the exception of 8265 and 976 lines, and the trend of slight decline in weight was maintained on next age groups. From leading purebred lines with the highest body weight at this age are animals of line 88550 (47.924kg) with proven differences compared with lines 1254, 913 ($P \leq 0.05$) and 8265 ($P \leq 0.001$). Proven differences are available for animals from line 3156 compared to 8265 ($P \leq 0.01$) and for originating from line 27 compared to 8265 ($P \leq 0.05$). It is striking that weaned with low body weight lambs from a breeding line 51 (with Chios blood), after elimination of maternal effect and likely heterosis effect manifestation, offset the weight development and on 9 months reached 47.40kg live-weight as are aligned and in some cases superior to their peers from purebred lines ($P \leq 0.01$ compared to line 942).

The tendency for superiority in weight development in them is maintained in later life. Sheep from line 51 are with the highest body weight on 1.5 years - 70.22kg, with proven differences compared with animals of all leading SPBM lines ($P \leq 0.01$, $P \leq 0.001$). On 2.5 years they again reached the highest live weight - 74.44kg as observed differences are proven to breeding lines 913, 6988 ($P \leq 0.05$), 88550, 3156, 976, 27 ($P \leq 0.01$) and line 522 ($P \leq 0.001$). The same age with the highest live-weight from pure-bred lines represent animals from line 1254 (72.54kg) with proven differences compared with lines 522, 976 and 27 ($P \leq 0.05$). For animals of line 8265 are available proven differences compared breeding lines 976 and 27 ($P \leq 0.05$).

The obtained values for weight development at different ages depending on the breeding line showed that SPBM sheep are characterized by high body weight, and the most stable in this regard are presented the sheep on line 51 (with Chios blood) and those from pure-bred SPBM lines - 27, 3156, 88 550 and 913. After weaning, inside-linear variation of the body weight follows declining trend, reaching 8.14% in 2.5 years.

Table 3. F –test of significance of the variance analysis of live weight in SPBM sheep

№	Sources of variation	Live weight			
		at weaning	on 9 months	on 1.5 years	on 2.5 years
1	Father line	n.s	+++	++	++
2	Mother line	n.s	+	n.s	++
3	Year of birth	+++	+++	+++	+++
Significance of differences at:		+ $P \leq 0,05$; ++ $P \leq 0,01$; +++ $P \leq 0,001$			

The analysis of variance reported high significant impact of the year of birth on the phenotypic expression of the trait live weight of all ages ($P \leq 0.001$) (Table 3). Stancheva (2003) in SPBM animals and Taskin et al. (2012) in sheep of synthetic dairy breed Sönmez also found significant effect of year of birth on sheep weight development. Father line has reliably high impact of the live weight at 9 months ($P \leq 0.001$) and less at 1.5 and 2.5 years of age ($P \leq 0.01$). The influence of the mother line of the studied trait was less for 9 months ($P \leq 0.05$) and 2.5 years of age ($P \leq 0.01$).

Table 4. Heritability (h^2) of the live weight in SPBM sheep

Live weight, kg	n	h^2	SE
at weaning	821	0,094	0,071
on 9 months	760	0,306	0,106
on 1.5 years	680	0,340	0,720
on 2,5 years	571	0,068	0,091

For study period is established low hereditary variance of trait live weight at weaning and at 2.5 years of age ($h^2 = 0.094$ and $h^2 = 0.068$) and moderately high for 9 months and 1.5 years ($h^2 = 0.306$ and $h^2 = 0.340$) (Table. 4). Received low estimate on h^2 for trait live weight at weaning - 0.094 confirms our opinion that this is not a manifestation of the genotype, but the logical consequence of allowing lambs for breeding with lower body weight at the discretion of the breeder, maternal effects and environmental factors. Slavov et al. (2008) reach a similar conclusion in a study of North East Bulgarian thin-fleece sheep breed. The established medium-high values for body-weight heritability on 9 months and 1.5 years - 0,306 and 0,340 were lower than those obtained in our study of the same flock at the time of population creation (Stantcheva, 2003). This, we believe is due to the interaction genotype - environment and changes in ontogenetic development.

Conclusions

- Body-weight on 9 months measured of the female lambs SPBM represents 65.65% of that of the animals of 2.5 years (70.97 kg), and 91.06% - of 1.5 years.
- The highest values of the coefficient of variation ($C = 21.31\%$) is a live weight of 100 days of age, and then in the other age groups in a declining trend, reaching 8.14% of 2.5 years.
- Values obtained for the weight development at different ages depending on the breeding line showed that SPBM sheep are characterized by high body weight, and the most stable in this regard are presented the sheep of line 51 (with Chios blood) and those from purebred SPBM lines - 27, 3156, 88 550 and 913.
- Year of birth has a high significant impact on the trait live weight of all ages ($P \leq 0.001$). The father line has a highly reliably effect of live weight at 9 months ($P \leq 0.001$) and less at 1.5 and 2.5 years of age ($P \leq 0.01$).
- The value of heredity of the live weight categories are of low to medium (0.094 to 0.340). The main reason for this may be given targeted selection, interaction genotype - environment and changes in ontogenetic development.

Acknowledgment

Research was part of the project Б 01/22 от 05.12.2012г. (2012-2014) "Development of DNA markers (CAST, MSTN) for fattening ability and meat quality in Synthetic population Bulgarian Milk, Karakachanian and Copper Red Shumen sheep breeds" financed by the Ministry of Education, Youth and Science, Republic of Bulgaria.

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EFFECT OF MANNAN-OLIGOSACHARIDE BIO-MOS ON LIPIDS IN THE SERUM OF SIMENTAL CALVESStolić N.^{1*}, Milošević B.², Spasić Z.², Ilić Z.², Pešić B.¹¹ College of Agriculture and food technology, Prokuplje, Serbia² Faculty of Agriculture, University of Pristina, Serbia

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Abstract

The effect of prebiotic preparation BIO-MOS („Altech", USA), based on mannan-oligosaccharide, on growing of suckling calves was investigated. In general, the results of the experiments showed that this prebiotic preparation for 1-3 month aged calves had stimulative effect on growth, improved the digestibility of nutrients and had positive effect on the immunological status of the animals. In this work the results of the change in total lipids in the calves blood serum are presented. The level of total lipids in the serum was analyzed after 30, 60 and 75 days of calves growth, both in the experimental and control group. The total lipids were 3.58 ± 0.803 g/l day 30, 3.58 ± 1.350 day 60, and there was a significant increase of this parameter at day 75 being 5.12 ± 0.732 . There was no statistically significant difference between the values obtained for the control group.

Key words: Prebiotic, calves, blood serum, lipids.

Introduction

Dairy calves are susceptible to many pathogens and nutritional problems during the first few weeks of life. For several years antibiotics have been used to overcome these problems, to obtain economic benefits in terms of improved calves performance and reduced medication costs. However, the use of antibiotics in animal husbandry can cause antibiotic resistance of pathogen bacteria. Thus, there is increasing interest in replacement of antibiotics with natural alternatives, such as probiotics, prebiotics and symbiotics in order to enhance animal performance and health (Ballou, 2011; Krol, 2011; Roodposhti and Dabiri, 2012). Probiotics are used to balance the host intestinal microbial eco-system and restore its resistance to diseases, by intake of viable probiotic microorganisms. On the other hand, prebiotics are non-digestible food ingredients that selectively stimulate the growth and/or activity of certain bacteria in the colon. It is known that prebiotics can have positive effect on various physiological processes and improve animal health, primarily by modification of the composition and functionality of gastrointestinal microflora. These processes include digestion, nutrient utilization, natural defence mechanism against pathogen and overall immunity system and finally, quality of animal products. Dietary prebiotics could have effect on lipid metabolism. It has been reported that consumption of prebiotics lower the concentration of both cholesterol and triglycerides (Roodposhti and Dabiri, 2012; Samanta et al, 2013). Among prebiotics mainly non-digestible oligosaccharides are ranked, such as mannan-oligosaccharides (MOS) – a complex

carbohydrates derived from cell walls of *Saccharomyces spp.* yeast. Mann-oligosaccharides have high resistance toward acidity and human gastrointestinal enzymes, and thus rich the colon undigested, being substrates for beneficial intestinal bacteria. On the other hand, they can reduce pathogen bacteria due to specific molecular features.

Fimbriae are rich in lectins and it is essential for bacteria adherence to the mannose-containing cells in the intestinal tract. Mannan-oligosaccharide provide a mannose-rich source for bacteria attachment, instead of being attached to the gut wall and cause disease. The selectivity of mannanoligosaccharides is based on that some beneficial bacteria (*Bifidobacterium longum*, *Lactobacillus casei*, *L. acidophilus*, *L. delbruckii*) produce the enzyme mannanase which targets its hydrolysis and therefore do not enable formation of complexes, unlike to those undesirable pathogen strains lacking the enzyme (Sharon and Lis, 1993; Fekete et al, 2002). Due to its beneficial effects, dietary intake of mannan-oligosaccharide can contribute to increase vitality of animals, enhancing both the digestive and immune system that further leads to optimum production and economic benefits.

The aim of this work to present the effect of dietary intake of prebiotic preparation BIO-MOS, based on mannan-oligosaccharide, which has been suggested as an effective prebiotic preparation, on lipids in the serum of growing of suckling calves.

Material and methods

The experiment was organized as a group control system, with calves of domestic spotted cattle in the type of Simmental. Calves were healthy, progressive, and vital without shortcomings and defects. Calves were housed in facilities for the calves, where they were located after the separation from their mothers. The first 5 days after receipt of colostrum they started receiving the mother's milk, and later consumed the cumulative milk. Calves were marked with tattoo stamps and numbered ear tags. Microclimatic conditions in the facilities during the experiments, keeping, and care, feeding and watering were identical for all calves. Two groups of calves were formed, a control group that was fed without added manan-oligosaccharide, and the experimental group, which received in milk the mentioned prebiotic. Each group of calves was comprised of 16 calves so that in total experiment was conducted with 32 calves.

At the start the calves were fed fresh colostrums. After the fifth day they were fed with 6 l of the cumulative milk produced on the farm. Treatment group was supplemented with 4 g prebiotic Bio-Mos/calf/day. The calves were fed via nipple bottle feeder, signed for each calf. After the day 15 a mixture was included in the daily diet according to the calves' physiological requirements and regulations on quality of animal food and feed in Serbia (Sl. List 20/2000, 38/2001). To avoid imprecise estimates, alfalfa flour was used up to 15 % in the mixture instead of hay. The blood samples were collected at the farm at defined time (day 30, 60 and 75) and analysed at the Institut of Veterinary of Serbia in Belgrade. Total lipid level was determined by the sulfo-phospho-vanillin color reaction, tryglyceride by GPO/PAP method and level of total cholesterol by CHOD/PAP, as described by Stolic, 2007 in more detail. Data were expressed as mean $\pm$ standard deviation for triplicate determinations and the statistical analysis was carried out using Install Prism4.

Results and discussions

The effect of prebiotic preparation BIO-MOS („Altech”, USA), based on mannan-oligosaccharide, on growing of suckling calves was investigated. In order to determine whether this prebiotic induces significant changes in the level of blood serum proteins and lipids, the biochemical analysis of the blood samples was performed after 30, 60 and 75 days of growth, both in the experimental and control group. The results of the level of total proteins, albumins and globulins are reported in our previous work, Stolić et al 2015.

Figure 1 shows the total lipids in the serum of the calves. The results showed that the level of the total lipids did not significantly changed the this period, being 3.58 ± 0.8 g/l at day 30, 3.58 ± 1.35 g/l at day 60, and 5.12 ± 0.73 g/l at day 75. There was no statistically significant difference between the experimental and the control group.

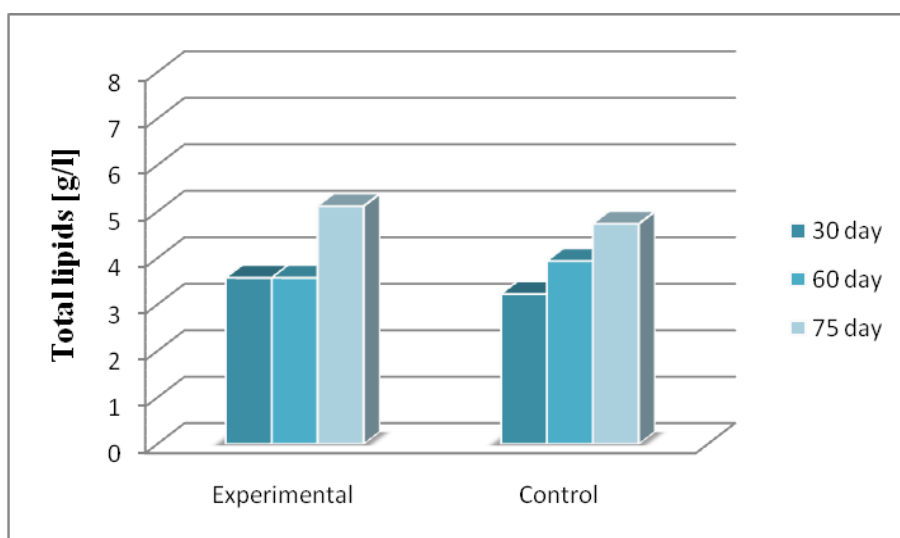


Figure 1. Level of total serum lipids in suckling calves.

As the level of total lipids did not change in response to the administration of the prebiotic, more detailed analysis was undertaken. The level of triglycerides and total cholesterol was examined in the blood samples. Figure 2 shows the level of triglycerides in the serum.

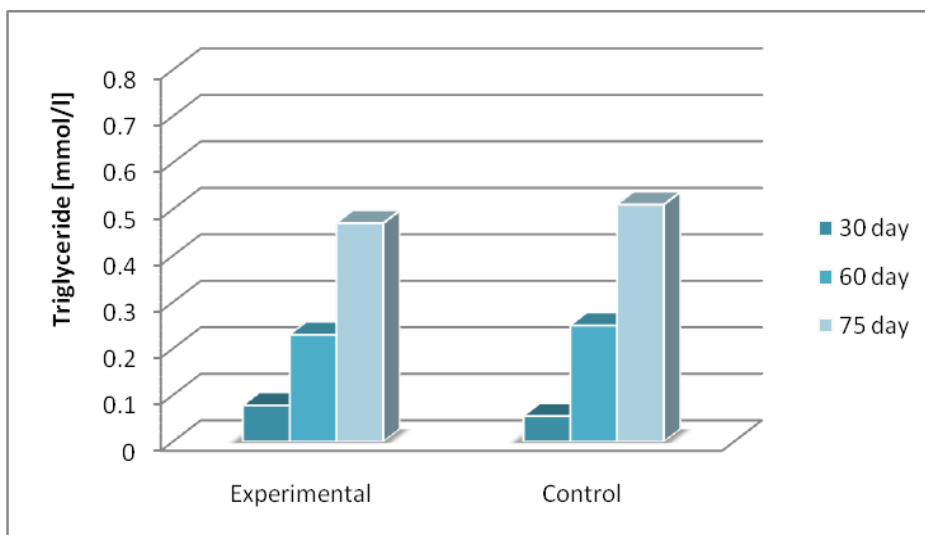


Figure 2. Level of serum triglycerides in suckling calves.

The serum tryglyceride in the experimental group were 0.25 ± 0.078 mmol/l, 0.23 ± 0.032 mmol/l and 0.47 ± 0.12 mmol/l at day 30, 60 and 75 respectively (Figure 2). It can be seen that serum triglyceride had normal level and also did not change in direct response to the prebiotic intake, but most likely due to animal normal diet and growth. Figure 3 shows the level of total cholesterol.

The level of total cholesterol was 2.23 ± 0.817 mmol/l at day 30, 1.82 ± 0.543 mmol/l at day 60 and 3.3 ± 0.45 g/l at day 75. There were no significant difference between the experimental and control group.

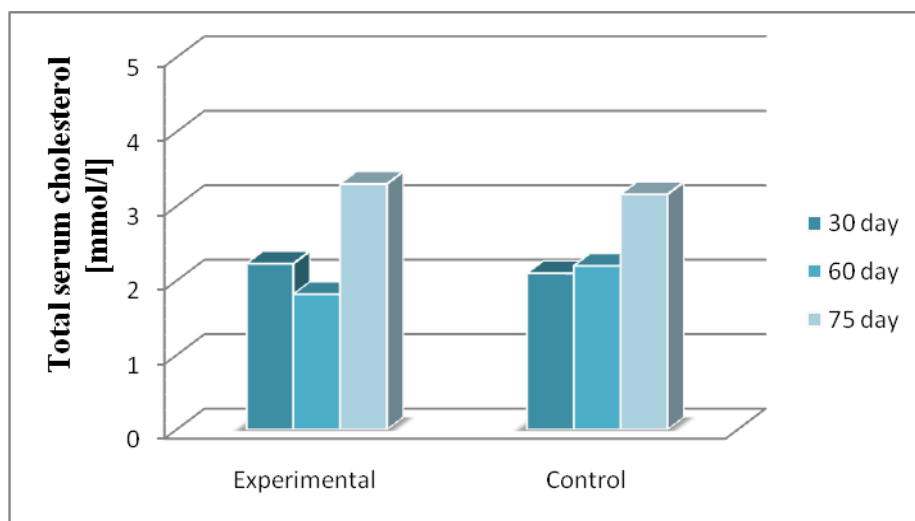


Figure 3. Level of total serum cholesterol in suckling calves.

Conclusions

The overall that this prebiotic preparation for 1-3 month aged calves had stimulating effect on growth, improved the digestibility of nutrients and had positive effect on the immunological status of the animals. However, the results of the blood serum analysis, including both proteins and lipids, show that the addition of the prebiotic Bio-Mos had no effect on these parameters. The change of total proteins and lipids are rather related to the normal diet and animal growth.

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OPTIMIZATION OF ENZYMATIC ASSAY OF SUPEROXID DISMUTASE AND GLUTATHION PEROXIDASE ACTIVITY IN MILK WHEYTrajchev M.^{1*}, Nakov D.¹, Gjorgoski I.²¹Faculty of Agricultural Sciences and Food, Institute of Animal Biotechnology, Department of Animal Health and Animal Welfare, University „St. Cyril and Methodius“, Skopje, Republic of Macedonia²Faculty for Natural Sciences, University „St. Cyril and Methodius“, Skopje, Republic of Macedonia

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Abstract

General spectrophotometric kinetic protocols are described to measure the antioxidant enzyme activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in milk whey. The SOD convert superoxide radical into hydrogen peroxide and molecular oxygen, while the GPx convert hydrogen peroxide into water. In this way, two toxic species, superoxide radical and hydrogen peroxide, are converted to the harmless product water. The applicability of this methods for milk samples from dairy cows were tested. SOD activities in the milk whey was determined by the autooxidation of pyrogallol in presence of DTPA in TRIS-HCl buffer, pH= 8.5. The effect of reaction conditions on enzymatic inhibition of pyrogallol autooxidation was studied when different amounts of sample were added in reaction mixture. The reaction mixture for determination of GPx activity contained EDTA, reduced glutathione, glutathione reductase, NADPH and cumene hydroperoxide in phosphate buffer, pH= 7.6. The effect of reaction conditions on enzymatic NADPH consumption was studied when different amounts of reduced glutathione and whey sample were added in reaction mixture. The both spectrophotometric methods were modified in order to accommodate kinetic analyses in 96-well micro plates. The rate of pyrogallol autooxidation and NADPH oxidation linearly depend from amount of whey sample in reactive mixture. Applying higher amounts of milk whey in reactive mixture was decreasing optical density reading of pyrogallol autooxidation at 415 nm and increasing the optical density readings of NADPH oxidation at 340 nm, in a linear way. The methods could be validly applied for milk whey samples.

Key words: dairy cows, milk whey, superoxid dismutase, glutathion peroxidase.

Introduction

Reactive oxygen species (ROS) are produced in many aerobic cellular metabolic processes. They include, but are not limited to, species such as superoxide and hydrogen peroxide which react with various intracellular targets, including lipids, proteins, and DNA (Bandyopadhyay et al., 1999). Although ROS are generated during normal aerobic metabolism, the biological effects of ROS on these intracellular targets are dependent on their concentration and increased levels of these species are present during oxidative stress.

Cells contain a large number of antioxidants to prevent or repair the damage caused by ROS, as well as to regulate redox-sensitive signaling pathways (Halliwell and Gutteridge, 2007). Three of the primary antioxidant enzymes contained in mammalian cells that are thought to be necessary for life in all oxygen metabolizing cells are superoxide dismutase (SOD), catalase, and a substrate specific peroxidase, glutathione peroxidase (GPx) (Halliwell, 1987). The SOD catalyze the dismutation of the superoxide radical anion into oxygen and hydrogen peroxide, while the catalase and peroxidases convert hydrogen peroxide into water and in the case of catalase to oxygen and water (Bordo et al., 2000). The net result is that two potentially harmful species, superoxide and hydrogen peroxide, are converted to water.

The oxidative stability of milk is a balance between pro- and anti-oxidative factors (Lindmark-Mansson and Akesson, 2000); hence, identification and characterization of these factors is necessary to predict the rate of oxidation. A number of low-molecular weight antioxidants have been described in milk, e.g., ascorbate, urate and atocopherol, to mention the most important as judged from their abundance and consumption during oxidation (Nielsen et al., 2001). However, milk also contains antioxidative enzymes, such as catalase, superoxide dismutase and glutathione peroxidase (GPx) that can reduce oxidative deterioration, but the importance and relative contributions of these enzymes to the oxidative stability of milk is unclear (Lindmark-Mansson and Akesson, 2000).

The indirect spectrophotometric assay has been developed for measuring SOD and GPx activity in plasma, erythrocyte lysates, tissue homogenates and cell lysates (Paglia and Valentine, 1967; Marklund and Marklund, 1974).

To some extent, the limited knowledge of the role of SOD and GPx in milk may be explained by the lack of suitable activity assays. Spectrophotometric kinetic assays were already successfully been applied for blood samples, we aimed to establish this tests for milk samples to evaluate it's relationship with occurrence of oxidative stress in dairy cows and herein report the limitations we observed for the application of the assays in milk.

Materials and methods

Milk (raw, i.e. unhomogenized and unpasteurized) was obtained from private dairy farm. Milk was defatted by centrifugation at 5000 rpm for 20 minutes at 4°C, and skim milk beneath the cream was gently poured into a clean container. Acid whey was prepared by treating the samples with 1M HCl to pH 4.6, followed by centrifugation (5000 rpm for 20 min at 4°C) in order to remove precipitated proteins. The pH was adjusted usually to pH 7.6 using 1M NaOH. The samples were stored in stoppered plastic tubes at -80°C. Before use the aliquots were thawed.

SOD activities in the milk whey was determined by the autoxidation of pyrogallol in presence of DTPA in TRIS-HCl buffer, pH= 8.5. The assay medium in a total volume of 200 µl contained 100 mM Tris HCl buffer, pH 8.5, 1mM DTPA (diethylenetriaminepentaacetic acid) in presence of 0.2 mM of pyrogallol. Pyrogallol (1,2,3-benzenetriolo) was dissolved in 10 mM HCl to obtain stock solution (2 mM) which was prepare always fresh. Measurement of SOD activity was carried out spectrophotometrically at 25°C by the method of Marklund and Marklund (1974) with some modifications in order to accommodate kinetic analyses in 96-well microplates (Gao et al., 1998). The reading of absorbance at 415 nm for 3 minutes was made in interval of 60 seconds after an induction period of some 10 seconds. The bovine Cu/Zn-SOD was used as

standard for generation of a standard curve, a range activity of 100 U/ml to 0.09765 U/ml. The values obtained for SOD activity in the milk sample were then calculated from the standard curves using logarithmic regression and were finally expressed as percentage of inhibition of the pyrogallol autoxidation rate. One unit of the enzyme activity is defined as the amount which produced 50% inhibition of pyrogallol autoxidation under the standard assay conditions. The effect of reaction conditions on enzymatic inhibition of pyrogallol autoxidation was studied when different amounts of sample were added in reaction mixture.

The principle of the coupled enzymatic assay first described by Paglia and Valentine (1967) and modified according Chen et al. (2000) in order to accommodate kinetic analyses in 96-well microplates. The reaction mixture for determination of GPx activity in a total volume of 200 μ l contained 50 mM potassium phosphate buffer, pH= 7.6., 5 mM EDTA (ethylenediaminetetraacetic acid), reduced glutathione (GSH), 1 U/ml glutathione reductase (GR), 0.25 mM NADPH. The measurements were carried out at two different concentrations of GSH i.e. 2 mM and 1 mM. Then cumene hydroperoxide ($\text{C}_6\text{H}_5\text{O}_2$) was added to initiate the reaction in a total volume of 30 μ l at 37°C. Stock solution of cumene hydroperoxide (9.9 mM) in 50 mM phosphate buffer, pH= 7.6. was prepare always fresh. The effect of reaction conditions on enzymatic NADPH consumption was studied when different amounts of reduced glutathione (GSH) and whey sample were added in reaction mixture. The assay temperature was 37°C. The reading of absorbance was made at 340 nm for 3 minutes in interval of 60 seconds after an induction period of some 10 seconds. One unit of GPx was defined as the activity that causes the formation of 1,0 μ mol/L NADP^+ from NADPH per minute at pH 7.6 at 37°C in a coupled reaction.

All chemicals were of the highest purity grade. TRIS, pyrogallol, KH_2PO_4 , EDTA and $\text{C}_6\text{H}_5\text{O}_2$ were purchased from Merck, Darmstadt, Germany. DTPA, Cu/Zn-SOD, GSH, NADPH and GR were obtained from Sigma Chemical Co., St. Louis, MO, USA. The absorption was determined with a spectrophotometer Bio-Rad 680 XR, microplate reader.

Results

The standard curves demonstrated a linear relationship between absorption measurements and the SOD activity. The colored dye is directly proportional to the percentage of the pyrogallol autoxidation rate. Since the rate change of absorbance per minute at 415 nm is proportional with the amount of superoxide anion, the SOD activity in reactive mixture as an inhibition activity of pyrogallol autoxidation is proportional with decrease in color development at 415 nm. Therefore, the increasing of absorbance at 415 nm (A_{415}) per minute followed a linear kinetics, showed on Figure 1.

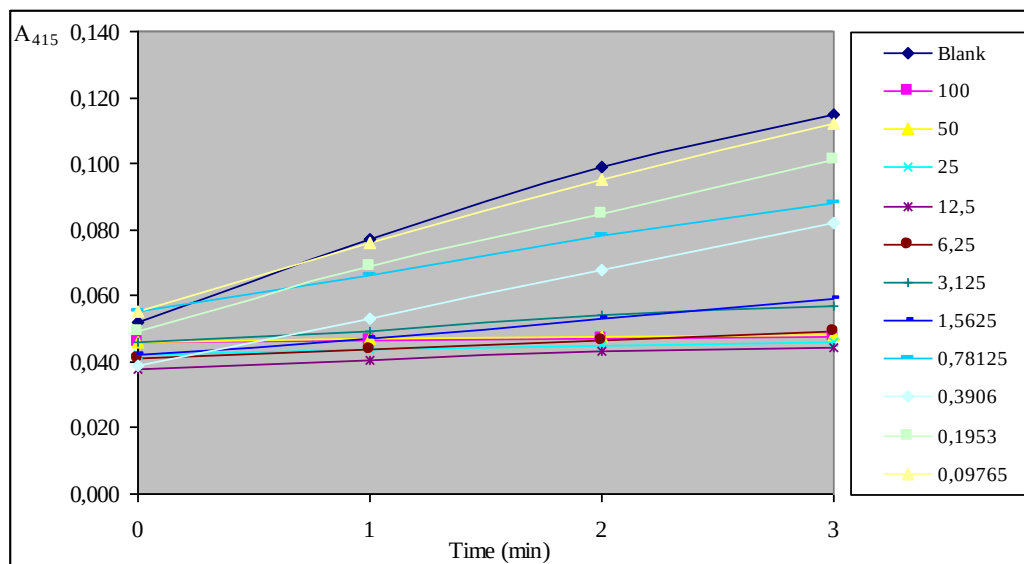


Figure 1. Linearity of standard curves using different concentrations of bovine Cu/Zn SOD ($\mu\text{U/ml}$)

On Figure 2 is showed standard curve of an SOD inhibition activity of pyrogallol autooxidation. The logarithmic function of standard curve was used for estimation of SOD activity in milk sample.

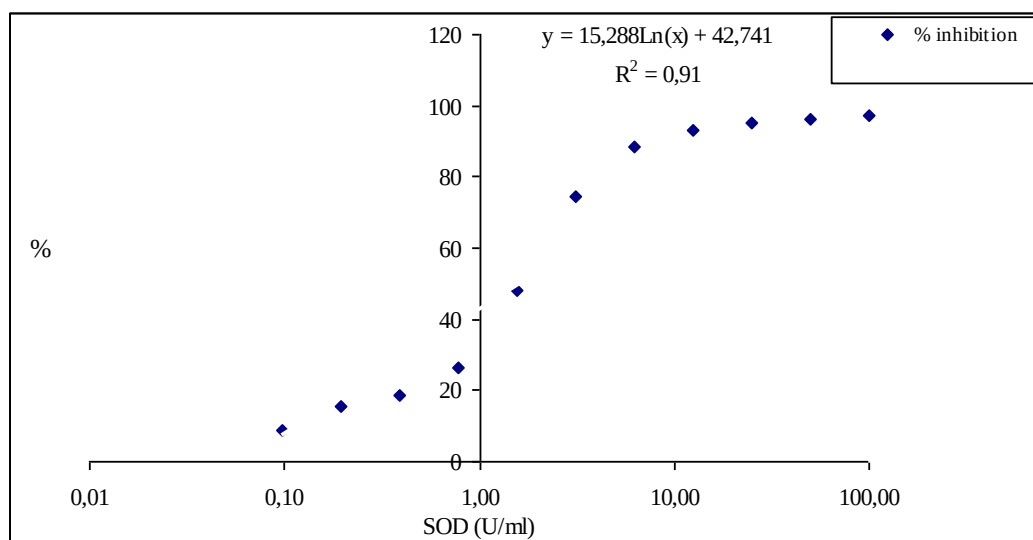


Figure 2. Representative standard curve using different concentrations of bovine Cu/Zn SOD. The regression equation was $y = 15.288\ln(x) + 42.741$ with $r = 0.91$

Using milk samples, the linearity of the assay was established in a range between sample dilution 1:40 to 1:2.5 (Figure 3).

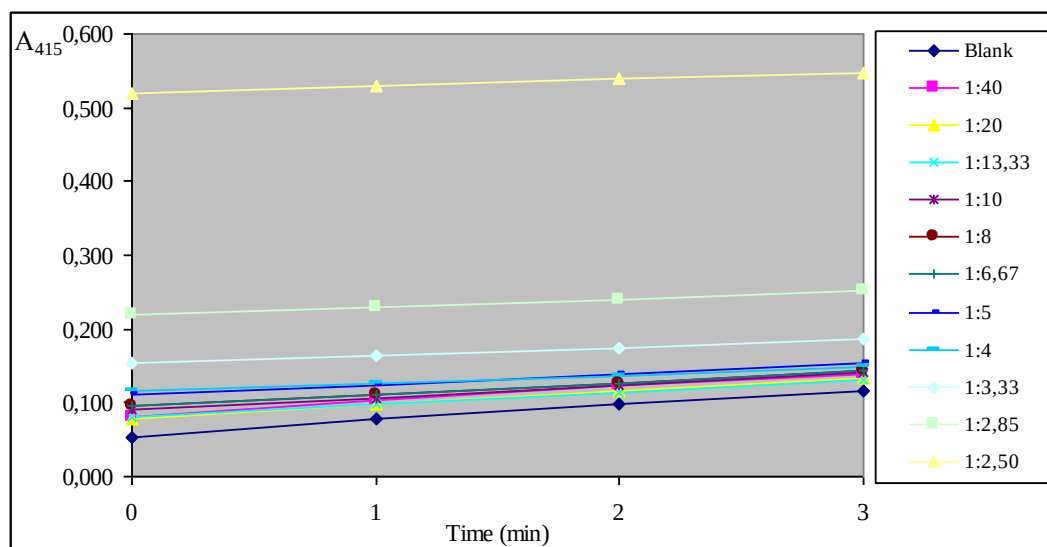


Figure 3. Evaluation of linear kinetic related to SOD activity using different volumes of milk serum

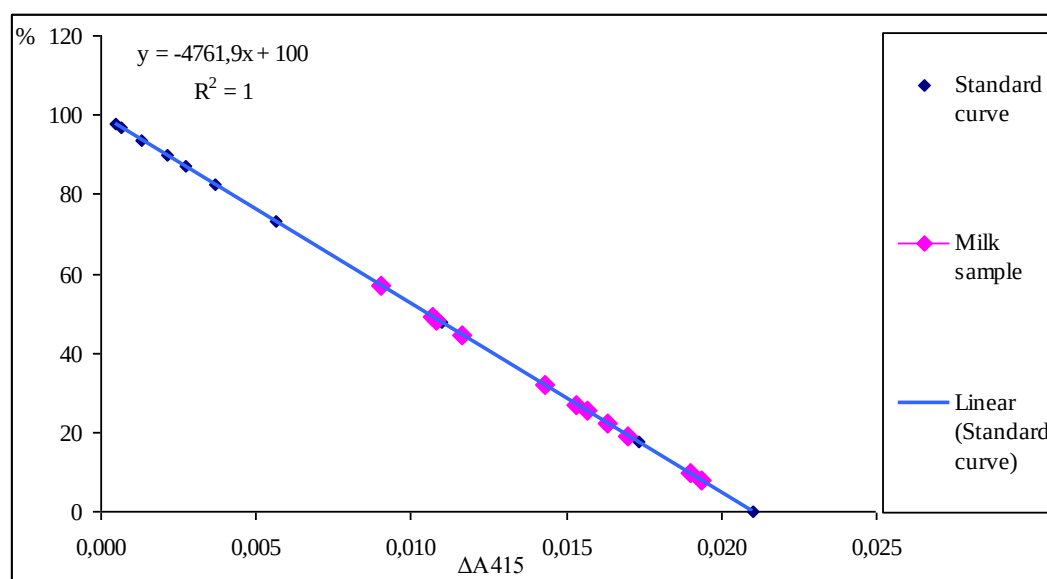


Figure 4. Net rate of absorbance change (standard curve vs. different dilutions of milk sample)

The limit of detection was defined as the concentration of bovine Cu/Zn SOD that yielded net rate of absorbance change different from the blank value measured by using H_2O and corresponds to the lowest standard concentration of 0.09765 U/mL SOD. The measured SOD activity in milk sample was in the range of standard curve which allow reliable measurements.

Figure 5 shows apparent rates of changes in absorbance at 340 nm as a measure of the oxidation of NADPH in the presence of milk, as a function of substrate concentration (GSH). The validity of this procedure is demonstrated by the constant relationship between decrease in absorbance at 340 nm per min and enzymatic NADPH consumption. Aimed to

get better linear kinetic in enzymatic NADPH oxidation, there was used different dilutions of milk sample and different concentration of GSH in reaction mixture. The linearity of the assay was established in a range between sample dilution 1:20 to 1:1000.

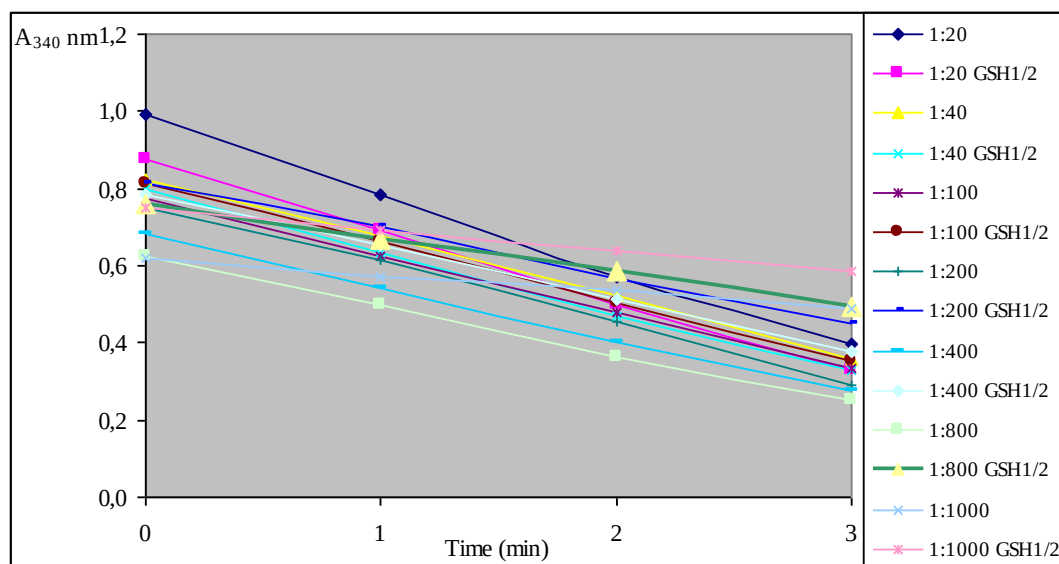


Figure 5. Evaluation of linear kinetic related to GPx activity using different volumes of milk serum

Discussion

Most procedures for determining SOD are based on the ability of the enzyme to inhibit oxygen-dependent reactions. Superoxide, produced by an *in vitro* reaction, reacts with the indicator giving absorbance at specific wavelength that increases with time. The correlation between the SOD concentration and the inhibition rates of the superoxide reaction with the indicator, allows to determine the enzyme activity. The inhibition of pyrogallol autoxidation brought about by SOD can be employed in a rapid and convenient method for the determination of the enzyme. After the above investigation we chose to use 0.2 mM pyrogallol in air-equilibrated 50 mM Tris-HCl buffer pH 8.5, containing 1 mM diethylenetriaminepentaacetic acid (DTPA).

Pyrogallol autoxidizes rapidly in alkaline aqueous solution, its autoxidation rate is measured from the linear increase in adsorbance at 415 nm for some minutes (usually from 1 to 3 minutes each assay). The increment of the band is linear within 7-10 minutes, then the colour turns green and finally, after a few hours, a yellow colour appears.

The increase in the absorbance at 420 nm after addition of pyrogallol was inhibited by the presence of SOD. As reported by Marklund and Marklund (1974), the rates of pyrogallol autoxidation were determined at pH 8.0 and 9.2. The rate of increase in absorbance at 420 nm per min was increased with increasing pH from 8.2-8.9. The autoxidation of pyrogallol was rapid at pH 9.2 but followed a non-linear pattern. By contrast, at pH 8.0 the autoxidation of pyrogallol was slow but followed a linear kinetics. We observed that better sensitivity and reproducibility were obtained when the pH of the assay mixture was kept at 8.5. At pH 7.9 the autoxidation is inhibited to 99% by Cu/Zn SOD, being the enzyme activity independent of pH in the range 5.5-9.5 (Gao et al., 1998).

The sensitivity to SOD decreases when the pH is increased, but still amounts to 93% at pH 9. At higher pH values there is a strong decrease in the sensitivity to SOD and at pH 10.6 the autoxidation is inhibited to only 15% by SOD (Violi et al., 1985).

Higher concentrations of pyrogallol decrease the sensitivity of assay (Gao et al., 1998). At 0.5 mM pyrogallol the sensitivity is decreased by a factor of 3.3. Iron, even in trace amounts, accelerates pyrogallol autoxidation. DTPA was found to prevent interference from Fe^{2+} (as well as from Cu^{2+} and Mn^{2+}) and was therefore chosen as chelator in the assay medium. In the absence of DTPA the autoxidation is faster than in its presence, and it tends to vary in rate and is less affected by superoxide dismutase. In the presence of DTPA the rate is independent of the concentration of the chelator, which indicates that the effect of DTPA is only due to its binding of traces of metal ions. Most measurements in the present report were performed in the presence of 1 mM DTPA. In the present method one unit corresponds to 100 ng bovine Cu/Zn SOD in a total volume of 1 ml; thus the method is about as sensitive as the method based on the reduction of cytochrome c by xanthine oxidase (Beyer and Fridovich, 1987). Using different amounts of the milk whey, the linearity was confirmed. This method is fast, cheap, simple, sensitive and requires common diagnostic tools as a commercial spectrophotometer featured by a visible light and very few amount of enzyme.

Glutathione peroxidase (GPx) activity has been measured through a coupled reaction with glutathione reductase (GR). In the assay, GPx reduce cumene hydroperoxide ($\text{C}_6\text{H}_5\text{O}_2$), and oxidize reduced glutathione (GSH) to oxidized glutathione (GSSG). The generated GSSG is reduced to GSH with through oxidation of NADPH to NADP^+ by glutathione reductase (GR). The decrease of NADPH is proportionally to GPx activity in the reactions. The decrease of NADPH can be easily measured by absorbance at 340 nm.

Chen et al. (2000) found that GPx activity in added whey or milk was found to increase as buffer pH increased from 7.0 to 8.0, and a pH of 7.6 was selected to avoid a high water-blank. The same authors reported When the phosphate concentration was changed from 25 to 100 mM/L, GPx activity decreased and the blank increased, showing that lower buffer concentration favored GPx activity. When the assay was performed at 25°C instead, the GPx activity in whey was 30% lower than at 37°C. According them, the GPx activity in milk was only slightly higher than that in whey indicating that most of the GPx in milk was recovered in whey.

The oxidation of NADPH was dependent from the presence of GSH and increased by the addition of milk. The better sensitivity and reproducibility were obtained when assay was performed in presence of 1 mM GSH. Usually for milk assays a lower GSH concentration, a lower peroxide concentration and a more alkaline pH were used than in blood assays (Debski et al., 1987; Bhattacharya et al., 1988; Avissar et al., 1991). One reason for this is probably that erythrocytes contain mainly cellular GPx and milk mainly extracellular GPx (Bhattacharya et al., 1988; Avissar et al., 1991; Lindmark-Maensson and Akesson, 2000). An important difference between these two forms is that extracellular GPx has a lower rate constant for GSH than cellular GPx (Esworthy et al., 1993) and at cellular levels of GSH (millimolar) the eGPx reaction appears to be up to 10-fold slower than the cellular GPx reaction. Moreover, cellular GPx could not be saturated by GSH and that a high GSH concentration could inhibit GR, thus decreasing the overall reaction rate.

Furthermore, there was dependence between substrate addition and presence of milk sample in reaction mixture, where the oxidation of NADPH clearly increased following addition of cumene H_2O_2 . Although the absorbance decreases during the assay

due to decreasing concentrations of NADPH, the negative rates have been converted to positive values throughout. Avissar et al. (1991) found the same GPx activity in milk using either H₂O₂ or tert-Butyl Hydroperoxide (t-BHPx) as substrate.

The presence of EDTA turned out to be critical for the apparent oxidation of NADPH by milk.

Contrary, some authors failed to detect any specific activity of GPx in bovine milk. Stagsted (2006) found GSH-independent NADPH oxidation in milk and GPx-catalysed t-BHPx - independent oxidation of GSH was observed. Author used 25 mM EDTA to allow optical measurements in concentrated milk samples. Although only 50% of the apparent turbidity could be eliminated with EDTA. Furthermore, they observed that concentrations of EDTA above 6 mM completely inhibited the apparent activity in milk. Therefore, we perform assay on milk whey, previously cleaned from turbidity and pH was adjusted to pH 7.6.

There are several reports on the activity of SOD and GPx in milk (Bhattacharya, et al., 1988; Debski et al., 1987; Avissar et al., 1991; Chen et al., 2000; Lindmark-Maensson and Akesson, 2000; Filipović et al., 2005; Kasapovic et al., 2005). According this reports, SOD activity in bovine milk was in the range from 0.92 to 3 U/ml. In raw cows' milk, extracellular form of GPx activity had demonstrated levels between 12 and 32 U/mL, and the activity has been found to be significantly correlated with selenium concentration (Przybylska et al., 2007). Debski et al. (1987) reported that GPx dependent peroxidase activity was approximately one-third of the total peroxidase activity and was found to be similar in human and bovine milk. However, some reports disagree regarding the measured activity of SOD and GPx in milk (Stagsted, 2006; Kovaceva et al., 2007).

Conclusions

The present study describes an spectrophotometric kinetic protocols to measuring the activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in milk whey. It has been shown that the linear kinetic observed in milk was depended from amount of whey sample and concentration of other compounds in reactive mixture. The both methods are reproducible and can be used for determination of antioxidant enzymes SOD and GPx in milk whey, suggesting that this antioxidative enzymes are of importance for milk stability and quality. SOD and GPx activity occurs in bovine milk, but their importance in this matrix is not well known.

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EFFECTS OF THE IMPLEMENTATION OF THE BREEDING PROGRAMS TO DEVELOP DAIRY CATTLE BREEDING IN VOJVODINA

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Abstract

The breeding programs for Holstein-Friesian and Simmental breed of cattle in Vojvodina defines breeding goals, population size, breeding methods, selection program and gene banks, research and development tasks to program efficiency increasing, the conditions for successful breeding of domestic animals and ensuring the dissemination of genetic progress and improve the quality of livestock products in accordance with the zootechnical standards. The legal basis for the breeding program is given in the Act of Livestock from 2009, while the first breeding programs came in 2010, and enforced to the end of 2014. Based on the current situation in the breeding of Holstein-Friesian and Simmental, breeding objective for controlled Holstein-Friesian breed population in Vojvodina is recording herds increase by 10%, average milk production in standard lactation over 7500 kg with 3.90% fat and 3.40% protein, and improve conformation; for the Simmental recording herds increased by 20%, average milk production in standard lactation over 5000 kg with 4.00% fat and 3.50% protein, as well as improved conformation (especially udders). On the basis of information from the Main breeding organization it was determined that the goal for Holstein breed was reached since recording herds increase by 30.85%, while the Simmental populations increase by 112.05%. Milk yield goal, for Holstein-Friesian breed has not achieved, but milk production increased by 10% compared to 2009. Milk yield goal, for Simmental breed is filled in the final year of programme implementation with production of more than 5000 kg. We conclude that the implementation of the major breeding programs gave a positive effect on the development of dairy cattle breeding in Vojvodina.

Key words: breeding programs, recorded herds, dairy cattle.

Introduction

Multiple role and importance of the dairy production demand system's sustainability and quality. Performance of dairy cattle, as the basic system component, we can improve by improving their genetic potential. The breeding program is a set of decisions and activities for, primarily, genetic improvement of the animals, so that they can better adapt to the environment or be competitive as much as possible. Organized breeding of farm animals in Vojvodina started by establishing the Regional Institute of Animal Science in 1950. Center for the breeding of farm animals at the Department of Animal Science, Faculty of Agriculture in Novi Sad, was registered in 2010., as the Main breeding organization in

Vojvodina by the Ministry of Agriculture of Republic of Serbia, since when began implementation of the breeding programs for Holstein Friesian and Simmental breed (Trivunovic et al., 2014). For the economic viability of dairy cattle, as the most important part of livestock production, it is necessary to clearly define the breeding goals and program and implement them (Bogdanovic et al., 2005). The breeding goal is a specific combination of economic values and genetic information of all the traits that we want to improve. Some of the most biologically and economically important traits of dairy cows are: a) production of large amounts of high value milk, b) reproductive efficiency, d) good health, c) the "ideal" conformation, d) longevity (Berry, 2014). Although the breeding goals vary in different situations, they are usually characterized by profit function which shows the direction in which genetic changes of particular traits affecting the cow's profitability.

Also, farm animal welfare has become increasingly important and relevant from the societal point of view (Oltenacu and Broom, 2010). Dairy industry success depends on the perception of its products and production methods by the public (Tonsor et al., 2009). Furthermore, breeding for an increased production level has led to a decrease of within-breed variability (Danchin-Burge et al., 2012). So, there are numerous reasons for the broad breeding goals (sustainability of production, consumer awareness about animal welfare, reduction of production cost) (Atsbeha et al., 2014). As the animal's performance for importance traits is greatly influenced by environmental (more than genetic) factors, sustainability and quality of dairy production systems requires optimization of environmental conditions by appropriate management practices on dairy farms (nutrition, health care programs, reproductive efficiency, etc.). The breeding programs can be considered as investment in the context of long-term development programs, and should be implemented in accordance with the national agricultural development strategy while respecting existing constraints (financing, etc.). It should be simple and reliable, at least initially, rather than sophisticated and sensitive to assumptions whose fulfillment cannot be guaranteed; with time and experience, the schemes may be more sophisticated (Kosgei et al., 2011). The above applies to all stages of the program definition and implementation. Analysis of implementation is an important component of breeding programs (FAO, 2010). Also, it is necessary to promote the breeding program and its results.

The breeding programs for Holstein-Friesian and Simmental breed of cattle in Vojvodina defines: breeding goals, population size, breeding methods, selection program and gene banks, research and development tasks to increase program efficiency, the conditions for successful breeding of animals, ensuring the genetic progress dissemination and improving the quality of animal products in accordance with the zootechnical standards (Main breeding program of Holstein-Friesian and Simmental cattle in Vojvodina, 2010). Subjects in the implementation of Main breeding programs of Holstein-Friesian and Simmental cattle in Vojvodina are: Farmers, Basics breeding organizations, Regional breeding organizations, Main breeding organization, Organizations with special authorization, Provincial Secretariat for Agriculture, Water Management and Forestry, Ministry of Agriculture and Environmental Protection. The most important objectives in the first breeding programs for Holstein Friesian and Simmental cattle (2010-2014), were: population increase by 10%, average milk production (305-d) over 7500 kg, fat content 3,90%, protein content 3,40%, and conformation improving, for recorded Holstein Friesian population; population increase by 20%, average milk production (305-d) over 5000 kg, fat content 4,00%, protein content 3,50% protein, conformation improving (especially

udders), for the recorded Simmental population. The aim of this paper is to examine the effects of the breeding programs implementation on the dairy production in Vojvodina.

Material and methods

Main breeding organization collect, process and analyze data related to recorded population. Data were collected from primary and regional breeding organizations, through quarterly reporting documentation and annual selection review which is obligatory event defined in the main breeding programs. Data for total population size and milk yield are obtained from Statistical Office of the Republic of Serbia; both data refers to the period 2010-2014. Number of cows and heifers in total and recorded population were calculated. Control of cows productivity is carried out by controlling the milk yield (Petrovic et al., 2006), conducted by authorized persons in accordance with methodology prescribed in the Main breeding programs of Holstein-Friesian and Simmental breed. All lactation records are standardized to 305-day, and lactation shorter than 200-day are excluded. The average milk yield is calculated both for recorded and total population. Yield and content of milk fat and number of complete lactation are calculated.

Results and discussion

Size of total and recorded Holstein Friesian and Simmental cows and heifers populations has increased, as well as share of recorded relative to the total population (Figure 1). The goal of increasing the recorded population was achieved for both breeds, since the recorded Holstein Friesian population increased by 30,85% and Simmental by 112,05%. This reflects the interest of farmers for the implementation of breeding programs on their farms. Also, it is a result of increased incentives for genetic improvement of livestock by the Ministry of Agriculture and Environmental Protection of Republic of Serbia. Given the total and recorded cows and heifers population size ratio, there is room for further increasing the share of recorded relative to the total population.

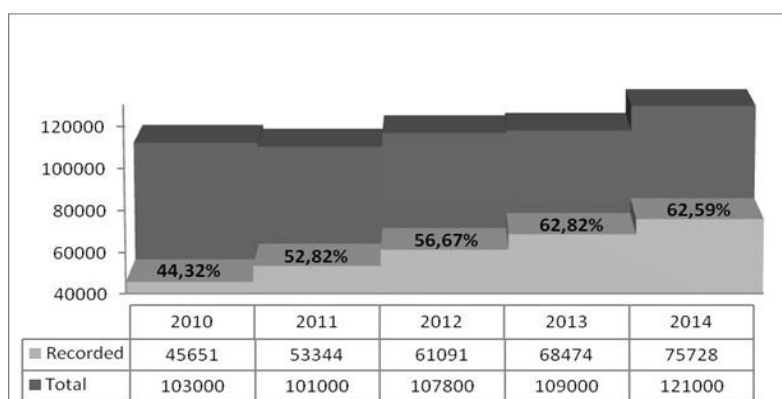


Figure 1. Size of the total and recorded cows and heifers population and share of recorded relative to the total population in the period 2010-2014.

In Croatia, the share of recorded relative to total population (2014.) is similar, 61,4% (CAA, 2015), while increasing the share of recorded relative to total population when comparing 2014. to 2010. is 18,3% (Serbia) and 7,8% (Croatia). In Slovenia (2013.), share

of recorded relative to the total population was 77,3%. In some other countries of Central and Eastern Europe share ranges from 77,8% in Austria to 93,9% in Czech Republic, while in the Poland is 30,5% (Bucek et al., 2014). The average milk yields in a recorded (HF, SIM) and total population (Figure 2), shows an increasing phenotypic trend of this very important production trait in recorded population, as well as higher absolute values, in contrast to total population, indicating the impact of implementation of breeding programs. However, although the average milk yield of Holstein Friesian population increased by 10%, the goal of 7500 kg of milk in 305-d lactation has not been achieved. The yield of more than 5000 kg of milk for the Simmental breed has been achieved in the last year of breeding program implementation.

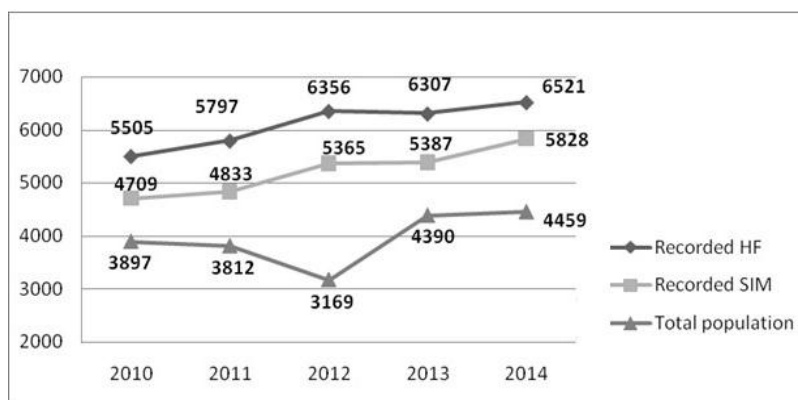


Figure 2. Phenotypic trends for 305-d milk yield of Holstein Friesian, Simmental and total population in the period 2010-2014.

Given the genetic potential of the Holstein Friesian cows for milk production, determined average milk yield in the Holstein Friesian population in Vojvodina is not satisfactory. But we need to know that the Holstein Friesian population in Vojvodina is very heterogeneous in many parameters (herd management, selection activities, herd size, etc.). In the former large state farms, for example, the average milk yield of Holstein Friesian cows are 8200 kg. The difference in the milk yield between Holstein Friesian and Simmental is also not satisfactory.

Table 1. Number of complete lactations, milk yield, milk fat content and yield in 305-d lactation of recorded Holstein Friesian and Simmental in the period 2010-2014.

Year	Holstein Friesian				Simmental			
	Number of lactations	Milk yield, kg	Fat, kg	Fat, %	Number of lactations	Milk yield,kg	Fat, kg	Fat, %
2010	17411	5505	202	3,36	2378	4709	181	3,52
2011	17307	5797	220	3,74	2738	4833	219	3,73
2012	18407	6356	241	3,8	3134	5365	215	4,01
2013	22432	6307	237	3,76	4411	5387	213	3,95
2014	22375	6521	252	3,87	4924	5828	236	4,05

Increase of milk yield in the period 2010-2014. is 1013 kg for the Holstein-Friesian and 1119 kg for the Simmental cows. Increasing the number of complete lactations is apparent also, for both breeds; 4964 more lactation of Holstein Friesian cows has been

completed in 2014. compare to 2010. and 2546 more lactation of Simmental cows has been completed in 2014. compare to 2010. Milk fat yield and content also shows increasing phenotypic trend in both breeds; milk fat yield was increased by 50 kg in the Holstein Friesian (fat content, 3,87% vs. 3,36%) and by 55 kg in the Simmental (fat content 4,05% vs. 3,52%). The average milk yield and milk fat content of Holstein Friesian cows are lower in relation to Croatia (7160 kg, 3,99%) (CAA, 2015), as well as in relation to Slovenia (7385, 3,97%) (Kmetijski Institut Slovenije, 2014.). The average milk yield of Simmental cows is higher in relation to Croatia (5030 kg, 4,01%), as well as in relation to Slovenia (5283 kg, 4,05%). In the region of ex Yugoslavia, the share of the recorded relative to the total population is increasing, as well as the average milk yield, while the total population size is decreasing, (Stokovic et al., 2007). New breeding programs (2015-2020.) and goals for Holstein Friesian and Simmental cattle in Vojvodina are broadened by including new traits; production (yield and content of milk protein), functional-fertility and health (percent calf crop, calving percentage, days open, calf sex, type of birth- single, twin, triplet, calf scoring, calving ease scores, calf mortality, somatic cell count), as well as longevity and conformation traits. Traits are measured according to ICAR (International Committee for Animal Recording) standards.

Conclusions

Dairy is a significant branch of agriculture and the overall economy in Vojvodina. Therefore it is of great importance to increase the number of the dairy cattle, as well as share or recorded relative to total population. This will increase the number of dairy cattle under the measures of breeding programs, primarily under the measures of genetic improvement. Proactive approach by all stakeholders will contribute achieving this goal, (e.g., state support for agriculture, improved breeding organizations, new technologies, promotion of breeding programs, etc.). This article just shows significant positive effects of implemetation of breeding programs for Holstein and Simmental cattle on the overall performance of dairy cattle. The milk yield and milk fat yield and content in a recorded population of Holstein Friesian and Simmental cows shows evident phenotypic increasing trend as opposed to a population that is not controlled. It actually indicates the great importance of implementation of breeding programs on the development of dairy cattle production in Vojvodina. Objectives in new breeding programs are broadened by including new traits (production, functional, conformation) which are recorded in accordance with professional standards. This, along with the optimization of management (nutrition, health care programs, housing, etc.) will further enhance and expand the positive effects of implementation of breeding programs for Holstein Friesian and Simmental cattle on development of dairy in Vojvodina. As the main goal of animal breeding is to improve genetic merit of the animals in the next generations, aim for the future is to estimate the genetic trends for traits of interest.

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THE USING OF BIOTECHNOLOGY IN ANIMAL BREEDING

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Abstract

This review focuses on recent developments applied in animal breeding including artificial insemination, embryo transfer, and embryo cloning and on approaches aimed at increasing production through use of pharmaceuticals and transgenesis. Livestock is becoming increasingly important to economic growth in developing countries and the application of biotechnology is largely dictated by commercial considerations and socio-economic goals. These technologies including artificial insemination, embryo transfer and other assisted reproductive methods are used in the genetic improvement of livestock. Biotechnology developments applied to livestock health, nutrition, breeding and reproduction are improving with a reasonable pace in developing countries. Simple bio-techniques such as artificial insemination have been well implemented in many parts of the developing world. However, advanced technologies including transgenic plant vaccines, marker assisted selection, solid state fermentation for the production of fibrolytic enzymes, transgenic fodders, embryo transfer and animal cloning are confined largely to research organizations (Onteru and et al., 2010). This paper reviews available biotechnologies with potential application in livestock improvement and identifies those which have been or may be applied in developing countries in general. The review covers biotechnology applications in the areas of animal genetics and breeding, including conservation of animal genetic resources, animal health, physiology of lactation and growth, and animal nutrition.

Key words: Turkey, Livestock production, Biotechnology.

Introduction

According to the estimation by Food and Agriculture Organisation of the United Nations (FAO), 70% of world population will be in hunger in 50 years from now (2060). It has been reported that the world human consumption for the animal protein was 29 g per capita daily (or 10 kg per capita consumption). Especially in the advanced countries, the human consumption was approximately 90 kg animal protein per capita consumption. However, the trend toward increased per capita demand for animal source foods is occurring primarily in developing countries (80% of world population). Through biotechnology, it has the potential to improve the productivity of animals via increase growth, carcass quality and reproduction, improved nutrition and feed utilisation, improved quality and safety of food, improved health and welfare of animals and reduced waste through more efficient utilisation of resources. Therefore, the biotechnology of livestock production is

growing faster than any other sectors; and by 2020 livestock is predicted to become the most important agricultural sector in terms of value-added commodity.

The world's population is expected to grow to 7.5 billion people in 2020 with most of this growth occurring in developing countries (IFPRI, 2001). As a result, demand for animal products is expected to grow tremendously (Bradford, 1999). In view of this, the methods of livestock production must change to allow for efficiency and improvement in productivity. Biotechnology (Biotech) is increasingly becoming a sustainable means of improving livestock production by directly influencing animal health, nutrition, reproduction, breeding and genetics (Bonneau and Laarveld, 1999).

Biotechnology will provide new and unprecedented opportunities to improve the productivity of animals through increased growth, carcass quality and reproduction, improved nutrition and feed utilization, improved quality and safety of food, improved health and welfare of animals, and reduced waste through more efficient utilization of resources. The livestock industry will benefit also from the application of biotechnology in other areas such as in the development of new and improved feedstuffs, as well as in microbiology as related to food and bioremediation (Bonneau and Laarveld, 1999).

Livestock is becoming increasingly important to economic growth in developing countries and the application of biotechnology is largely dictated by commercial considerations and socio-economic goals. These technologies have been used in the genetic improvement of livestock, particularly in cattle and buffaloes, and the economic returns are significant (Madan, 2005).

Widespread use of some biotechnologies in animal husbandry resulted (Van Vleck, 1981) and will result in a major impact on genetic progress. Different types of biotechnologies may have influence on both animal breeding and the resulting genetic progress through three main ways (Gengler and Druet, 2001)

- Biotechnologies can affect efficiency of reproduction and therefore also selection programs: artificial insemination, embryo transfer, sexing, cloning and other related techniques (e.g., Ruane and Thompson, 1991; van Vleck, 1981).
- Biotechnologies can improve determination of genetic values of animal: genetic markers, candidate genes and other related techniques (e.g., Georges et al., 1995; Renaville et al., 1997).
- Biotechnologies can transform artificially the genome at the DNA level: genetic engineering, gene transfer and related techniques (e.g., Solter, 1981).

This paper reviews biotech in livestock production citing practical examples while focussing on possibilities for application in animal production

Reproductive animal biotechnology

Various biotechnology methods are used in improving the breeding stock of animals. These include artificial insemination (AI), embryo transfer (ET), in-vitro fertilization (IVF), somatic cell nuclear transfer, and the emerging technology on somatic cell nuclear transfer.

Artificial Insemination (AI)

One of the earliest perfected technology is Artificial Insemination (AI) where new breeds of animals are produced through the introduction of the male sperm from one superior male to the female reproductive tract without mating. AI reduces transmission of venereal

disease, lessens the need of farms to maintain breeding males, facilitates more accurate recording of pedigrees, and minimizes the cost of introducing improved genetics (Wilmut et al.1979). Various technologies have evolved that led to the efficient use of AI in developing desired livestock, including the methods of freezing semen or cryopreservation and sperm sexing.

AI technology use is still more generally associated with dairy cattle than other domestic livestock species. The limitations of AI use in beef cattle include the difficulty in detecting heat in large beef herds kept on ranches and the less frequent handling of individual cows. In sheep and goats the failure to develop a simple, non-surgical insemination procedure has prevented extensive exploitation of the technology in sheep (Robinson and McEvoy 1993).

In-vitro Fertilization

With in vitro fertilization (IVF), a technician removes unfertilized eggs (oocytes) from the donor cow's ovaries, usually recovering 6-8 useable oocytes. The oocytes mature in an incubator and are fertilized with sperm. The resulting zygotes incubate and develop in the laboratory before being placed into the recipient cow. While IVF can produce many fertilized embryos, the added expense of ET makes the procedure prohibitive in most cases (Becker and Covan, 2009).

In case other artificial reproductive techniques fail due to difficulties such as blocked reproductive systems, non-responsive ovaries in the females, marginal semen quality and quantity in the male, and presence of disease, in vitro fertilization (IVF) is used. The fertilization of the sperm and the egg is conducted in vitro (outside the animal's body) at specific environmental and biochemical conditions. To date, successful IVFs have been conducted in various animal species due to advances in embryo production and cryopreservation of reproductive cells. Since the birth of the first rabbit conceived through IVF in 1959 (Chang, 1959), IVF offsprings have been born in mice, rats, hamsters, cats, guinea pig, squirrels, pigs, cows, monkeys, and humans (Bearden and Fuquay, 2000)

Embryo Transfer (MOET)

After AI and estrus synchronization, embryo transfer (ET) is the third most commonly used biotechnology. In ET, a donor cow of superior breeding is hormonal induced to superovulate. The eggs are then fertilized within the donor, the embryo develops and is then removed and implanted in a recipient cow. Between removal and implantation, embryos may be frozen for safekeeping. Because of the relatively high costs, ET is used mostly within registered cow herds (Becker and Covan, 2009).

Embryo transfer (ET) from one mother to a surrogate mother makes it possible to produce several livestock progenies from a superior female. Selected females are induced to superovulate hormonally and inseminated at an appropriate time relative to ovulation depending on the species and breed. Week-old embryos are flushed out of the donor's uterus, isolated, examined microscopically for number and quality, and inserted into the endometrium of the uterus of surrogate mothers.

By increasing the number of offspring that can be obtained from monotocous species in particular, MOET has the potential to increase genetic improvement by enhancing the selection intensity on the female side (Georges, 1994)

Multiple Ovulation and Embryo Transfer (MOET) is a well established technology and is used to obtain over 80% of the embryos produced for commercial purposes (Thibier,

2001). Multiple ovulation embryo transfer (MOET) is a composite technology which includes superovulation, fertilisation, embryo recovery, short-term in vitro culture of embryos, embryo freezing and embryo transfer. Benefits from MOET include increasing the number of offspring produced by valuable females, increasing the population base of rare or endangered breeds or species, ex situ preservation of endangered populations, progeny testing of females and increasing rates of genetic improvement in breeding programmes (Rege, 1994).

It was suggested that application of MOET in nucleus breeding schemes could increase animal genetic gains by 30-80% (Nicholas and Smith 1983). More recently it has been concluded that the earlier figures were over-predictions (Keller et al 1990). The over-predictions arose partly because the assumed average number of progeny (eight) per donor female was unrealistically high and partly because of wrong assumptions made about genetic parameters (Keller et al 1990).

ET increases reproductive rate of selected females, reduces disease transfer, and facilitates the development of rare and economically important genetic stocks as well as the production of several closely related and genetically similar individuals that are important in livestock breeding research.

Somatic Cell Nuclear Transfer

Somatic cell nuclear transfer (NF) is a technique in which the nucleus (DNA) of a somatic cell is transferred into a female egg cell or oocyte in which the nucleus has been removed to generate a new individual, genetically identical to the somatic cell donor (Tian et al, 2003). This technique was used to generate the sheep Dolly from a differentiated adult mammary epithelial cell which demonstrated that genes that are already inactivated in differentiated tissues can be completely reactivated (Wilmut, 1979). NF technology creates possibility of generating clones from superior genotype and can be used to efficiently evaluate effects of genotype x environment interactions and testing or dissemination of transgenics. Problems on high rate of pregnancy loss, survival of newborn and increased incidence of abnormal development due to incorrect reprogramming of nuclear DNA (epigenetic inference) and unusual conditions during in-vitro processes make this a pre-commercial technology.

Cloning

Cloning is a biotechnology developing rapidly and with significant public controversy. Most people think of cloning as the creation of an organism that is genetically identical to another one. However, scientists use the term more broadly, to refer to production not only of such organisms but also of genetically identical cells, and to replication of DNA and other molecules. It also refers to a form of reproduction found naturally in many single-celled organisms, as well as plants and animals. Those differences in meaning and usage have caused some confusion in public debate about cloning, where the main area of controversy relates to artificial cloning involving higher organisms, including humans (Johnson and Williams, 2006)

Sexing

Sexing of semen and of embryos provides in species and production circumstances where one sex is preferred, a way to produce the wanted type of animal (Betteridge et al., 1981; Johnson et al., 1998). Sexing of embryos is a technique that is rather reliable nowadays and

currently used on a rather large extent. Some progress was recently made in the field of sexed semen. One problem is the price of sexing and the eventually reduced fertility of this semen. The other issue is that sexing has to be economic what means that the extra-price to be paid for sexed semen has to be in relation with the extra income (Gengler and Druet, 2001) .

Animal genetics and breeding

Genetic improvement of livestock depends on access to genetic variation and effective methods for exploiting this variation. Genetic diversity constitutes a buffer against changes in the environment and is a key in selection and breeding for adaptability and production on a range of environments. In developed countries, breeding programmes are based upon performance recording and this has led to substantial improvements in animal production (Rege, 1994).

Indicator traits

Selection is the process breeders use to produce genetic change, realizing that genetic change and genetic improvement are not necessarily the same. Producers can change many traits genetically but change does not necessarily mean improvement. Improvement implies the production of superior animals and for livestock production the definition of superior animals are those with greater profitability. This manual explains underlying genetic mechanisms, concepts of selection, and tools that can be used to make better selection decisions to help producers meet their goals. The assumption throughout is that the goal of sire selection and beef enterprises is profitability. The difference between indicator and economically relevant traits (ERT) and the ability to distinguish between the two are keys to improving profitability. By identifying the economically relevant traits, selection focus can be narrowed, resulting in faster genetic improvement and improved profitability. In the end, the goal of focusing selection on ERT is to increase the probability that breeders will make selection decisions that make them more profitable (Enns, 2010).

Indicator traits are characteristics which are genetically correlated to traits of economic importance and are easier to measure than the latter. Such traits are usually not the target of genetic improvements but provide an indirect means of improving a targeted trait. Blair et al (1990) reviewed some physiological and/or metabolic characteristics which might be considered as potential indicator traits. Indicator traits can improve genetic response by increasing accuracy of selection and reducing generation interval. The value of an indicator trait will depend largely on the magnitude of co-heritability (square-root of the product of heritability of the indicator and of the target trait) and the genetic correlation between the two traits (Woolliams and Smith 1988). Woolliams and Smith (1988) concluded that, with high co-heritability, selection for the indicator trait alone can result in greater rates of response than is possible with progeny testing, especially when breeding values are not accurately measured by progeny testing.

Transgenic animals

There are various definitions for the term transgenic animal. The Federation of European Laboratory Animal Associations defines the term as an animal in which there has been a deliberate modification of its genome, the genetic makeup of an organism responsible for inherited characteristics (FELASA, 1982). A transgenic animal is an animal whose hereditary DNA has been augmented by addition of DNA from a source other than

parental germplasm through recombinant DNA techniques. Transfer of genes or gene constructs allows for the manipulation of individual genes rather than entire genomes. There has been dramatic advances in gene transfer technology in the last two decades since the first successful transfer was carried out in mice in 1980 (Palmiter et al 1982; Jaenisch 1988). The technique has now become routine in the mouse and resulting transgenic mice are able to transmit their transgenes to their offspring thereby allowing a large number of transgenic animals to be produced. Successful production of transgenic livestock has been reported for pigs, sheep, rabbits and cattle. The majority of gene transfer studies in livestock have, however, been carried out in the pig. Although transgenic cattle and sheep have been successfully produced, the procedure is still inefficient in these species (Niemann et al 1994). Transgenesis offers considerable opportunity for advances in medicine and agriculture. In livestock, the ability to insert new genes for such economically important characteristics as fecundity, resistance to or tolerance of other environmental stresses would represent a major breakthrough in the breeding of commercially superior stock. Another opportunity that transgenic technology could provide is in the production of medically important proteins such as insulin and clotting factors in the milk of domestic livestock (Rege, 1994).

Conclusions

As a result, some of biotechnological methods are used effectively to increase animal production today while some of them have a chance for the future application. Today, animal products has strategic importance and more effective and widely using of these biotechnological methods are necessary in animal production

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STUDY ON EWE LIVE WEIGHT OF PATCH FACED MARITZA SHEEP BREED

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Abstract

The aim of this study was to investigate magnitude of live weight of adult ewes and rams of Patch-faced Maritza sheep breed as well as changes in ewe live weight from lambing to the end of lactation. Live weight of 296 ewes and 7 rams of Patch-faced Maritza sheep breed has been measured by a portable electronic scales FX2. The weighing was made in 6 herds in Plovdiv region (central part of South Bulgaria) with dried of ewes. In order to establish changes in ewe live weight during lactation an experiment was conducted with monthly weighing of 67 ewes in the herd of Agricultural university in Plovdiv. It was found that overall mean of ewe live weight is 74.47 kg and ram live weight in this study was 121.47 kg. In comparison with other local sheep breeds in Bulgaria and other European country this is higher live weight. ANOVA table showed a statistically proven impact of environmental factors - herd, age and body condition score of the ewe with high probability ($p < 0.001$). The changes of ewe live weight after parturition, were studied through monthly weighing after lambing to drying of the ewes. After parturition, ewe live weight decreased to 2nd month with 19.54 %, then to the end of lactation live weight is maintained at the same level with minor fluctuations.

Key words: live weight, adult ewes and rams, environmental factors, changes

Introduction

After 1990, great changes in the Bulgarian sheepbreeding have been occurred. The selection and genealogical structures of many existence sheep breeds have been changed. A lot of merino and semi-merino sheep breed lost of its relevance. Many sheepbreeders have preferences to local sheep breeds with better productivity – Pleven blackhead sheep, Stara Zagora sheep, White Maritza sheep, Patch-faced Maritza sheep, Elin-Pelin sheep. This poses serious challenges to the breeding work with these breeds. In order to survive these breeds need to be competitive to introduced sheep breeds. Developing sustainable breeding programs for local Bulgarian sheep breeds will contribute to the conservation of their competitiveness. New studies are needed for the magnitude of the productive and functional traits of local sheep breeds (milk yield, prolificacy, growing ability of the lambs, litter weight and live weight in different ages) in order to estimate better their potential to satisfy farmer's economical interest.

Milk yield and prolificacy of present population of Patch-faced Maritza sheep have been object of research in many other papers (Dimov and Djorbineva 1997, Dimov et al.,

1997, Dimov 1998, Dimov 2000, Dimov and Kuzmanova 2006). Live weight of Patch-faced Maritza sheep has not been studied yet.

As a dual purpose sheep breed, live weight of Patch-faced Maritza sheep has significant contribution to meat production because of which are needed new data and research.

Visual appraisals of Patch-faced Maritza sheep characterized them as large breed (Dimov 1998, 2010). Studies on live weight of adult ewes and rams of Patch-faced Maritza sheep breed has not been done till now if it does not take into account the study of Balevska and Tanev (1958), where the authors have measured live weight of local sheep – 40.05 kg (for period 1948 – 1955). However, these data are not relevant for today's population of Patch-faced Maritza sheep.

Breeding activities with Patch-faced Maritza sheep today pass off entirely with herds in private sector, because of which at this stage for full characteristic of the breed new data of live weight of different age categories are needed.

The aim of this study was to investigate magnitude of live weight of adult ewes and rams of Patch-faced Maritza sheep breed as well as changes in ewe live weight from lambing to the end of lactation.

Material and methods

Live weight of 296 ewes and 7 rams of Patch-faced Maritza sheep breed has been measured by a portable electronic scales FX21 Electronic Weighing System produced by ICONIX. The weighing was made in 6 herds in Plovdiv region for the period 15th August – 15th September 2008. In addition an experiment was conducted with monthly weighing of 67 ewes in the flock of Agricultural university in Plovdiv in order to find changes in live weight of the ewes from lambing to the end of lactation. It was covered the period from the last month of pregnancy to the end of lactation. Body condition score was measured at every weighing through system described by Todorov (1995). To take into account environmental effects fixed linear model was used:

$$Y_{ijkmn} = \mu + a_i + b_j + c_k + d_m + e_{ijkmn}$$

where:

Y_{ijkmn} = n-th measuring of j-th ewe in i-th herd, k-th age category and m-th body condition score.

μ = LS – mean of the population

a_i = fixed effect of the herd (1.... 6)

c_k = fixed effect of age of the ewe (1.....6)

d_m = fixed effect of body condition score (1....3)

e_{ijkmn} = residual error

To solve the equations of fixed linear model, statistical package of Harvey (1990) was used.

Results and discussion

The live weight is important functional trait in sheep, which influences meat and wool productivity, as well as milk production. In this study it was found overall mean of ewe live weight - 74.47 kg with low coefficient of variation. This is higher live weight in

comparison with other local sheep breeds. Ram live weight in this study was 121.47 kg, which present 162.66 % than ewe live weight. Although comparatively low number of the rams in this study, the results can be considered informative, against the background of the live weight of the ewes.

Table 1. Overall means and standard deviation of live weight of Patch-faced Maritza ewes and rams.

Traits	n	$\bar{x}$	Sd	Cv
Ewe live weight, kg	296	74.47	14.87	10.01
Ram live weight, kg	7	121.14	22.26	18.36

Legend: n – number of observations; $\bar{x}$ -overall mean; Sd – standard deviation; Cv –coefficient of variation.

It is well known that on a population level live weight as many other traits in sheep is influenced by too many environmental factors. By these reasons, many authors considered, that the overall means are not sufficiently informative for proper interpretation of the data obtained on a population level (Tzvetanov, 1990). Therefore, assessing the impact of environmental factors has become a common procedure in handling database of traits in farm animals.

ANOVA table showed a statistically proven impact of factors included in the model - herd, age and body condition score of the sheep at a relatively high probability (table 2).

Table 2. ANOVA of ewe live weight Patch-faced Maritza sheep breed.

Sources of variation	df	SS	MS	p
Herd	5	14822.35	2964.47	***
Age	5	5516.27	1103.25	***
Body condition score	2	6450.42	3225.21	***
Residual	283	15737.66	55.61	
Total	296			

Legend: df – degree of freedom; SS – sum of square; MS – mean square; p - probability *** = p<0.001

Review of the literature about Bulgarian sheepbreeding shows that only Stantcheva (2003) find a higher ewe live weight on a synthetic population in the country – 83.97 kg at 2.5 years of age and 87.51 kg for ewe 3.5 years. However, it has to keep in mind that these data Stantcheva (2003) found only in one flock, that is in sheep farm in Agricultural institute in Shumen. It is logical to assume that high live weight of synthetic population found of Stantcheva (2003) is influenced by the factor herd, because as it is well known that sheep farm in Agricultural institute in Shumen is one of the best in Northern Bulgaria and high level of nutrition is provided.

Most of the studies on the live weight of different sheep breeds and crosses in the country (Venev et al., 1974, Djorbineva, 1984, Tsvetanov, 1989, Georgiev, 1990, Dimov 1995) are implemented in one herd. Although data were collected in different farming years the herd effect is not taken into account by the authors. According to us when doing breed characteristic on a given trait, it must be done at least in several herds in order to be taken into account the herd effect, which in most cases is significant. This is an occasion to look at our results presented in table 3. If we consider LS-means in different herds it can be seen that herd effect causes variation in ewe live weight of Patch-faced Maritza sheep breed from 68.21 kg to 90.82 kg. This variation can be explained by the provided level of feeding and keeping of the sheep and the level of management in the herds. It could seek

other reasons for this difference, but at this stage in view of the fixed sources of variation that is the only explanation.

Table 3. LS-means и SE of live weights of adult ewes of Patch-faced Maritza sheep breed depending of the herd influence.

Names of owners of the herds and location	n	LS-means	SE
1. A. Gishin - Saedinenie	56	90.82	1.10
2. Agricultural university - Plovdiv	115	68.21	0.86
3. P. Dashev – Radinovo	36	75.29	1.32
4. R. Borisov- Manolsko Konare	54	76.34	1.05
5. J. Vitkov – Manolsko Konare	16	81.43	1.93
6. V. Rumenov	19	78.69	1.78
LS – mean	296	78.46	0.62

Legend: n – number of observations; LS – least square; SE – standard error

A number of studies on the ewe live weight of the other local sheep breeds as Black-head Pleven and Stara Zagora and some dairy crosses (Dzhorbineva , 1984, Tsvetanov , 1989, Georgiev , 1990, Dimov 1995) refer only to two age groups - 2.5 and 3.5 years. This is due to the old system of selection in the country, which was based on data for productive and functional traits only during the first two lactations (to 3.5 years of age). Former large cooperative farms from 1000 - 3000 ewes on the farm (before 1990) made it possible to form large flocks of the same age. The present situation in sheepbreeding is quite different. Our observations in a number of private farms have shown that there are many cases where the ewes in flocks of Patch-faced Maritza sheep are left for breeding purposes to 6.5 years of age and in some cases older. Our results show that the live weight of adult ewes increases to 6.5 years and reached 84.22 kg (table 4). This gives us reason to think that analysis of data on ewe live weight should be the basis of all age groups in the flocks. Otherwise, the information of the ewe live weight is not so accurate especially for older categories.

Table 4. LS-means and SE of ewe live weight at Patch-faced Maritza sheep breed depending of the age, kg

Age, year	n	LS-means,	SE
1.5	55	69.70	1.17
2.5	78	75.78	0.92
3.5	60	79.95	1.04
4.5	46	79.38	1.17
5.5	28	81.75	1.48
6.5	29	84.22	1.47
LS – mean	296	78.46	0.62

Legend: n – number of observations; LS – least square; SE – standard error

Ewe live weight was measured between 15th august and 15 september. This is a period when the ewes are dried off. Some of the ewes were pregnant, but this fact was ignored because they were in too early stages of pregnancy (1st -2nd month of pregnancy). However, the ewe live weight was influenced by body condition in this period. Ewes with a high milk yield during milking period had depleted body reserves and in worse body condition than those with a lower milk yield, which results in a lower live weight. Therefore, body condition has to take into account for a proper assessment of ewe live

weight. Restoration of body reserves of high yielding ewes takes about two months, so at the end of lactation (august-september) they are not reimbursed.

Depending of body condition scores, ewe live weight vary from 70.12 до 87.85 kg, which emphasize the great importance of assessing the body condition of the ewes when live weight is measuring (table 5).

Table 5. LS-means и SE of live weight of Patch-faced Maritza sheep depending of body condition score

Body condition scores	n	LS-means	SE
to 3.0	60	70.12	1.05
from 3.0 to 3.5	185	77.42	0.69
over 3.5	51	87.85	1.24
LS – mean	296	78.46	0.62

Legend: n – number of observations; LS – least square; SE – standard error

The ewe live weight is not constant. Depending on changes in the physiological condition ewe live weight is changing. After drying off the ewes and their mating, ewe live weight gradually increases until the end of pregnancy. After parturition, in connection with lactation and intensive use of body reserves to maintain energy balance the ewes lose a certain percentage of their live weight (table 6). It can be seen in the table 6, that after parturition ewe live weight decreased to 2nd month with 19.54 %, then to the end of lactation ewe live weight is maintained at the same level with minor fluctuations.

Table 6. Changes in ewe live weight after parturition during lactation.

Traits	Last month of pregnancy	Months of lactation					
		1st	2nd	3th	4th	5th	6th
Live weight, kg	83.95	70.38	67.55	68.32	69.44	65.58	68.08
Lost of weight, %	100	-16.16	-19.54	-18.62	-17.28	-20.69	-18.90

Conclusions

Based on this study it can be done the following conclusions:

Ewe live weight of Patch-faced Maritza sheep breed is 78.46 kg (LS-mean), which defines Patch-faced Maritza sheep breed as a large breed with a high live weight. Ram live weight of rams is 121.14 kg

Significant effect on ewe live weight have the factors - heard, age and body condition възраст и телесно състояние.

Live weight loss after parturition until the second month of lactation reaches 19.54% . After the second month until the end of lactation ewe live weight has a minor fluctuation.

Phenotypic characteristics of sheep breeds on the base of live weight of adult animals must be done based on measurement of the trait in several herds and possible inclusion of ewes of all ages. When live weight is measuring it should be take into account also body condition scores.

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UDC: 637.2(497.7)
Original scientific paper**DYNAMIC AND COMPOSITION OF LACTIC ACID BACTERIA (LAB) DURING
TRADITIONAL BUTTER AND BUTTERMILK PRODUCTION**Santa D.^{1*}, Srbinovska S.¹, Trajkovski G.²¹Faculty of Agricultural Sciences and Food - Skopje, University Ss. "Cyril and Methodius"
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Abstract

In rural areas of the Republic of Macedonia, traditional production of butter and buttermilk obtained from sour milk is very common practice with the long history. However, research on these dairy products has not been done before. The aim of this paper was to determine the composition and dynamics of lactic acid bacteria, during production of traditionally produced butter and buttermilk. Examined variants were produced from raw sheep milk at three individual households, over two years. The microbiological biodiversity during technology process of butter and buttermilk is described in this paper. During production 128 isolates of cocci and rod-shaped lactic acid bacteria were collected. Lactic acid bacteria isolated from investigated products were determined as: (*Cit*⁻) *Lactococcus lactis* ssp. *lactis*, *Streptococcus salivarius* ssp. *thermophilus*, (*Cit*⁺) *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*, *Lactobacillus delbrueckii* ssp. *bulgaricus* и *Lactobacillus plantarum*. The results showed that LAB in raw milk as starting material were in range between $1,2 \times 10^4 - 1,1 \times 10^6$ cfu/ml, and after fermentation and cooling their number increased to $8,3 \times 10^5 - 1,3 \times 10^7$ cfu/ml. In the final product LAB number was $1,2 \times 10^6 - 7,3 \times 10^6$ cfu/g and $2,3 \times 10^7 - 6,9 \times 10^7$ cfu/ml in butter and buttermilk respectively.

Key words: butter, buttermilk, traditional, lactic acid bacteria

Introduction

Traditionally milk and dairy products have always been one of the basic diets, where butter and buttermilk have special place and history in our region. Traditional production of butter from sour milk is still present in some rural households.

Lactic acid fermentation is a biochemical process caused by lactic acid bacteria (LAB), which are natural environment of milk and dairy products (Carr et al., 2002). Lactic acid bacteria (LAB) are widely distributed in nature, they are characterized as group of gram-positive, non-spore forming, cocci or rods, which produce lactic acid as the major end product during the fermentation of carbohydrates. LAB are best known for their use as starter cultures in the manufacture of dairy products such as acidophilus milk, yogurt, buttermilk, cottage cheese, hard cheeses (Carr et al., 2002).

LAB species belong to numerous genus under the family of *Lactobacillaceae* and the most common LAB cultures used in yoghurt manufacture are *Streptococcus thermophilus* and

Lactobacillus bulgaricus (Yantyati et al., 2014). Both types of LAB can exist in the milk as a monoculture, but their symbiotic relationship and their common activity during the milk fermentation affect its acidification, the texture development, viscosity and the creation of flavor and aroma components (Angelov et al., 2009). With regard to the production of lactic acid, *Lb. delbrueckii* ssp. *bulgaricus* produce more acid than other lactic acid bacteria (Wheater, 1995).

In sour milk other LAB can be also present, such as (*Cit*⁻) *L. lactis* ssp. *lactis*, which are present in large number and are very active, responsible for the "natural souring" or development of natural acidity (Foster et al., 1961).

Milk and cream are examples of *fat-in-water* (or oil-in-water) emulsions, where the milk fat exists as small globules or droplets dispersed in the milk serum. Milk fat is not a good environment for the microflora development, but in the butter among other bacteria, LAB can be also present. Hammer (1948) has isolated (*Cit*⁻) *L. lactis* ssp. *lactis* in butter and cultured buttermilk.

Bille (2013), in his study examined presence of the LAB in the traditional fermented product Omashikva in Namibia, which is similar to the production of traditional buttermilk. The predominant species were *Lactobacillus plantarum*, then (*Cit*⁻) *L. lactis* ssp. *lactis*, (*Cit*⁺) *L. lactis* ssp. *lactis*, with slightly lower presence of *St. Thermophilus* and some species of the genus *Leuconostoc*.

The microbiology of Leban Zeer or Kishk in Egypt, which is a similar product to traditional buttermilk, were studied by Morcos et al., (1973), Beuchat (1983) and Tamime and O'Connor (1995). They identified that the most dominant lactic acid bacteria were (*Cit*⁻) *L. lactis* ssp. *lactis*, *Lb. brevis*, *Lb. casei* and *Lb. plantarum*.

The present study is focused on the isolation, identification and dynamic of the dominant lactic acid bacteria during the production stages of traditional butter and buttermilk.

Materials and methods

In this study butter and buttermilk produced from the sour sheep milk was examined. Traditionally, butter is produced by churning of sour milk in traditional wooden hand churn, named butin (Figure 1). The samples for microbiological examinations were collected during two years (July and August-in the first year and July, August and September - the second year). Taking into account the duration of the technology process the samples have been taken in two days (Table 1).

Microbiological analysis

For determination of the number of rod-shaped LAB, MRS agar was used and the samples were incubated in anaerobic condition at the temperature of 30°C and 45°C for 72-96 hours, while for cocci LAB M17 agar was used and the samples were incubated in aerobic condition at the temperature of 30°C and 45°C for 48 hours. For the isolation and determination of cocci and rod shaped LAB was used M 17 broth and MRS broth.

Determination of the LAB was performed according to methods described in "Bergey's Manual of determinative bacteriology" (1948, 1994).

The following morphological characteristics are examined:

- Shape, size and color of the colonies (24, 48 and 72 hours after their cultivation on certain temperature on MRS agar);

- Shape and arrangement of the bacterial cells (after 24 hours);
- Gram staining.

Table 1. Stages of microbiological samplings

<i>I (First) day</i>
1. Raw milk 1
2. Boiled milk 1
3. Starter culture (from previous production)
4. Fresh fermented milk
<i>II (Second) day</i>
5. Sour milk after cooling
6. Raw milk 2
7. Boiled milk 2
8. Bulk fermented milk after churning (1 and 2)
9. Raw butter
10. Washed Butter
11. Buttermilk



Figure 1. Traditional hand churn (butin)

The following physiological characteristics were examined:

- Catalase determination (after 48-72 hours cultivation at the optimum temperature with 1 - 2 ml of 3% solution of H₂O₂);
- Testing of homo or hetero fermentation bacteria (after separation of CO₂ from glucose by the method of Gibson & Abd - el Malek and NH₃ formation of the hydrolysis of arginine);
- Growth on 15°C and 45°C (at 15°C during 21 days and at 45°C for 15 days on MRS agar);
- fermenting of certain carbohydrates, such as:
 - LAB rod-shaped - sucrose, lactose, maltose, mannitol and raffinose;
 - LAB cocci - sucrose, lactose, maltose and raffinose (cultivation within 14 days on nutritional substrate with corresponding carbohydrate and bromocresol purple as an indicator, with durham tubes used to detect CO₂ formation).
- Growth in salt presence (the MRS agar nutrient substrate added in 2, 4 and 6,5 % NaCl).

Results and discussion

During the traditional butter and buttermilk production 128 isolates of LAB were isolated and 38 isolates from total were selected for determination according the production stages (Table 2):

SECTION 1. ANIMAL BIOTECHNOLOGY

Table 2. Isolated and separated LAB according to the stages and days

Isolated LAB by stages		Determined LAB by stages	
<i>I (First) day</i>	<i>Isolates</i>	<i>I (First) day</i>	<i>Isolates</i>
Raw milk 1	11	Raw milk 1	4
Boiled milk 1	7	Boiled milk 1	2
Starter culture (from previous production)	10	Starter culture (from previous production)	3
Fresh fermented milk	13	Fresh fermented milk	3
<i>II (Second) day</i>	<i>Isolates</i>	<i>II (Second) day</i>	<i>Isolates</i>
Sour milk after cooling	10	Sour milk after cooling	4
Raw milk 2	15	Raw milk 2	4
Boiled milk 2	9	Boiled milk 2	2
Bulk fermented milk after churning (1 and 2)	15	Bulk fermented milk after churning (1 and 2)	5
Raw butter	11	Raw butter	3
Washed Butter	12	Washed Butter	3
Buttermilk	15	Buttermilk	5
<i>Total:</i>	<i>128</i>	<i>Total:</i>	<i>38</i>

Six of 38 isolates were hetero fermentative LAB and as technologically not significant they were not a subject in our research. Other 27 homo fermentative LAB isolates belonged to cocci, and 5 isolates were defined as rod shaped LAB. According determination and their properties, isolated bacteria belong to the species listed in Table 3 and 4.

Table 3. LAB species -cocci isolated by stages

LAB (cocci)	Number of LAB isolates	Stages of separation
(Cit ⁻) <i>Lactococcus lactis</i> ssp. <i>lactis</i>	11	In all stages (from 1 - 11 stage)
<i>Streptococcus salivarius</i> ssp. <i>thermophilus</i>	8	1, 2, 3, 4, 5, 6, 7 and 11 stage
(Cit ⁺) <i>Lactococcus lactis</i> ssp. <i>lactis</i>	5	8, 9, 10 and 11 stage
<i>Lactococcus lactis</i> ssp. <i>cremoris</i>	3	1 and 6 stage
<i>Total:</i>	<i>27</i>	

Table 4. LAB species rod-shaped isolated by stages

LAB (rod-shaped)	Number of LAB isolates	Stages of separation
<i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i>	4	3, 4, 5 and 11 stage
<i>Lactobacillus plantarum</i>	1	8 stage
<i>Total:</i>	<i>5</i>	

The dynamic of LAB during all stages of butter and buttermilk production are given in Table 5. Taking into consideration that the production of traditional butter is two days, in the discussion some stages are treated as single phase (1st and 6th stage represent the raw milk from first and second day and 2nd and 7th stage represent boiled milk).

Table 5. Dynamic of LAB in I-st and II-nd year according to the days

I day					
Sample from:	I / July	II / July	I / August	II / Aug.	II / September
Raw milk 1	$1,1 \times 10^6$	$8,2 \times 10^5$	$1,7 \times 10^5$	$1,0 \times 10^5$	$1,3 \times 10^5$
Boiled milk 1	$1,0 \times 10^4$	$9,2 \times 10^4$	$1,3 \times 10^3$	$1,1 \times 10^3$	$2,3 \times 10^4$
Starter culture (from previous production)	$1,2 \times 10^6$	$1,6 \times 10^6$	$4,6 \times 10^6$	$1,0 \times 10^6$	$1,3 \times 10^6$
Fresh fermented milk	$8,1 \times 10^6$	$2,3 \times 10^6$	$5,5 \times 10^6$	$1,8 \times 10^6$	$2,2 \times 10^6$
II day					
Sample from:	I / July	II / July	I / August	II / Aug.	II / September
Sour milk after cooling	$1,3 \times 10^6$	$9,2 \times 10^5$	$1,1 \times 10^6$	$8,3 \times 10^5$	$1,3 \times 10^6$
Raw milk 2	$6,1 \times 10^5$	$6,2 \times 10^5$	$1,2 \times 10^4$	$5,2 \times 10^4$	$1,5 \times 10^5$
Boiled milk 2	$7,2 \times 10^4$	$1,3 \times 10^4$	$1,1 \times 10^4$	$1,6 \times 10^4$	$1,3 \times 10^5$
Bulk fermented milk after churning (1 and 2)	$3,3 \times 10^6$	$1,6 \times 10^7$	$1,5 \times 10^6$	$5,4 \times 10^6$	$8,7 \times 10^6$
Raw butter	$3,1 \times 10^5$	$3,6 \times 10^5$	$1,4 \times 10^5$	$2,8 \times 10^5$	$7,0 \times 10^5$
Washed Butter	$1,1 \times 10^6$	$1,1 \times 10^6$	$8,3 \times 10^5$	$7,0 \times 10^5$	$9,0 \times 10^5$
Buttermilk	$4,8 \times 10^6$	$2,5 \times 10^7$	$2,4 \times 10^6$	$6,6 \times 10^6$	$9,3 \times 10^6$

In raw milk (1st and 6st stage), (*Cit*⁻) *L. lactis* ssp. *lactis*, *St.thermophilus* and *L. lactis* ssp. *cremoris* were isolated, with the dominant prevalence of (*Cit*⁻) *L. lactis* ssp. *lactis*.

This domination of LAB cocci is expected as they are characterized with more active metabolism and more intense lactose fermentation than rod shaped LAB (Alonso-Calleja et al., 2002, Mirzaei, 2011). As a result of LAB cocci activity, pH and oxygen presence are decreased, which leads favorable environment for more intensive development of rod shaped LAB (Ferrazza et al., 2004). *L. lactis* ssp. *lactis* in the ewe milk during the traditional production of beaten cheese was the dominant in the research of Levkov (2013) with 36% of the total population of cocci LAB.

According Delves-Broughton (1990), more dominant presence of (*Cit*⁻) *L. lactis* ssp. *lactis* is explained with the fact that these bacteria produce nisin, polycyclic antibacterial peptide consisted of 12 amino acid. Nisin inhibits the growth of certain cocci and rod shaped LAB, then staphylococci, propionic bacteria, some gram positive bacteria, yeasts and fungi reducing their presence in milk (Atte von Wright, 2012). This may be a reason that *L. lactis* ssp. *cremoris* is present in lower percent than (*Cit*⁻) *L. lactis* ssp. *lactis*.

Dynamic of LAB count was in range of $1,2 \times 10^4$ cfu/ml in July - $1,1 \times 10^6$ cfu/ml in August (Table 5). The average LAB count in raw milk ($3,76 \times 10^5$ cfu/ml) and is higher than the results of Desmaures et al. (1996), which is probably due to different origin of milk, hygiene, location and feed. Similar results were reported by Kakurinov (1997) where the number of LAB on cow milk ranged from $4,1 \times 10^6$ to $1,4 \times 10^7$ cfu/ml. The same author in another examination Kakurinov (2002) counted number of LAB from $1,0 \times 10^5$ to $2,5 \times 10^7$ cfu/ml. Levkov (2013) in her research on 2 variants of the ewe milk found that LAB cocci ranged from $1,08 \times 10^7$ to $1,22 \times 10^7$ cfu/ml for the 1-st variant and from $6,5 \times 10^5$ to $7,1 \times 10^6$ cfu/ml for the 2-nd variant while rod-shaped LAB in the 1-st variant ranged from $1,04 \times 10^6$ to $3,6 \times 10^6$ cfu/ml and in the 2-nd variant from $1,69 \times 10^6$ to $4,36 \times 10^6$ cfu/ml.

Boiled milk (2nd and 7th production stage) contained the same lactic acid bacteria as raw milk, but with a reduced number. Hammer (1948) has noted that *St. thermophilus* can easily be found and isolated from pasteurized milk, and Foster et al. (1961) stated that

(Cit⁻) *L. lactis* ssp. *lactis* can survive pasteurization of milk if it is in a higher number, which is usually the case, therefore these bacteria are often found in pasteurized milk. Total number of LAB in boiled milk ranged from $1,1 \times 10^3$ - $1,3 \times 10^5$ cfu/ml or an average $3,69 \times 10^4$ cfu/ml that indicates a decrease of LAB in terms of raw milk. The identified differences in the degree of reduction of LAB probably is a result of the milk heating temperature and the tolerance of microorganisms to the stress condition (Parente and Cogan, 2004), as well as additional contamination from dairy equipment (Manolopoulou et al., 2003).

As starter culture for traditional butter production, buttermilk from previous production was added. In this 3rd phase the presence of *St. thermophilus* was dominant, compared to *Lb. delbrueckii* ssp. *bulgaricus* and (Cit⁻) *Lact. lactis* ssp. *lactis*, with slightly lower percentage than the previous stages. According to Stević (1962), *St. thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus* are most prevailing species in the culture used for milk fermentation in households, known as “potkvas”. Вишневецкий (1955) has isolated the two types of bacteria in the starter culture that was used for production of *razenka* - Ukrainian sour milk. Additionally he found that the ratio of bacteria between *St. thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus* was 4:1-5:1, which differs from our research where the ratio was from 2:1 up to 3:1. During the production of traditional Turkish yayik butter made from yoghurt, *St. salivarius* ssp. *thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus* were predominant in the yoghurt as a start material with 41,2% of the total isolates with ratio almost 1:1 or 21,2%:20% (Sağdıç et al., 2002).

It is characteristic that the sturter culture (potkvas) in all trials in our research had a very minor differences in the prevalence of LAB, from $1,0 \times 10^6$ - $4,6 \times 10^6$ cfu/ml, or in average $1,94 \times 10^6$ cfu/ml.

In the 4th stage (fresh fermented milk) the same microorganisms as in sturter culture were isolated and the most dominant bacteria was *St. thermophilus*. In this stage was noted more intensive development of LAB. In July the number of LAB increased from 17.39 to 120 times compared to boiled milk, in August of 1636-4231 times, and in September for 96 times. The average increase was 1 220 times. According Вишневецкий (1955) after completed fermentation of Ukrainian yogurt *razenka*, the ratio between *St. thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus* was 40: 1-50: 1, which was not a case in our research where this ratio was from 5:1 to 10:1. This differences in the data is probably a result of a different technological process and sturter culture (potkvas) used.

The fermentation process is done by the activity of *Lb. delbrueckii* ssp. *bulgaricus* and *St. thermophilus*, through which lactose is transformed into lactic acid (Stević, 1962). It is known that in this process *St. thermophilus* contributes to creation of uniform, homogeneous and good consistency of sour milk and yogurt and prevents of serum separation. However, *Lb. delbrueckii* ssp. *bulgaricus* besides lactic acid formation, by the synthesis of acetaldehyde creates a pleasant and characteristic flavor in final products. Jespersion cited by Gruev (1979) isolated *Lb. delbrueckii* ssp. *bulgaricus*, *Lb. casei*, (Cit⁻) *Lt. lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris* from sour milk. The author noted that isolated rod shaped LAB can degrade more casein (25-30%) into soluble compounds, than isolated LAB cocci (15-17%). The amount of free amonoacids is increasing that result with higher nutritional value of yogurt.

In the sour milk after cooling (5th fase) the same microorganisms as in the previous two stages were isolated, but the ratio between *St. thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus* is quite reduced (2:1). The increased presence of *Lb.*

delbrueckii ssp. *bulgaricus* is due to the environmental conditions in the media. According to (Angelov et al., 2009) the more intensive oxygen use, the reduction of pH and redox potential by *St. thermophilus* stimulated and facilitated growth of *Lb. delbrueckii* ssp. *bulgaricus*, which have a greater capacity to produce more lactic acid than *St. thermophilus*.

Total LAB in the sour milk after cooling were $8,3 \times 10^5$ - $1,3 \times 10^7$ cfu/ml, which indicates some slight reduction compared to the previous stage.

In the bulk fermented milk after churning (8th production stage), (*Cit*⁻) *L. lactis* ssp. *lactis*, (*Cit*⁺) *L. lactis* ssp. *lactis* and *Lb. plantarum* were isolated, with domination of (*Cit*⁻) *L. lactis* ssp. *lactis*. The presence of *Lb. plantarum* is probably a result of the additional input from the wooden tools (hand churn). The same conclusion reported Manolopoulou et al., (2003). The number of LAB compared to the sour milk after cooling increased insignificantly (in average 6,89 times).

In the raw butter (9th stage), (*Cit*⁻) *L. lactis* ssp. *lactis* and (*Cit*⁺) *L. lactis* ssp. *lactis* were isolated and (*Cit*⁻) *L. lactis* ssp. *lactis* was more prevalent. Eighty-five LAB isolates were obtained from the yayik butters (traditional Turkish butter made from yoghurt) and the most frequent lactic acid bacteria were *Lactobacillus* sp. with 32 isolates and *Streptococcus* sp. with 22 isolates (Sağdıç et al., 2002). In another examination with traditional fermented milkbutter (Dhan) collected from individual households in West Algeria dominated lactic acid bacteria especially the genera *Lactobacillus*, *Lactococcus* and *Leuconostoc* with dominant *Lb. delbrueckii* ssp. *lactis* and *Lactobacillus plantarum* of *Lactobacillus* and *Lactococcus lactis* ssp. *lactis* of *Lactococci*. (Bettache et al., 2012). These results are not in accordance with our results, due to origin of milk and differences of technology.

In this stage (raw butter) slight reduction is noticed and the number of LAB was in the range of $1,4 \times 10^5$ - $7,0 \times 10^5$ cfu/g. Similar results from $0,6 \times 10^4$ - $5,55 \times 10^5$ cfu/g had (Idoui et al., 2010) with examination of samples of traditional produced butter from cow milk in Eastern Algeria. Almost same results were the results from traditional made butter produced in the rural area (Djelfa) in Algeria and that number ranged from $2,8 \times 10^5$ to $5,4 \times 10^5$ cfu/g (Mourad et al., 2014), with dominant *Lactobacillus* sp. (75%) according to *Lactococcus* sp. (25%). Bernard cited by Hammer (1948) reports the presence of (*Cit*⁻) *L. lactis* ssp. *lactis* in the butter, but (*Cit*⁺) *L. lactis* ssp. *lactis* can be also present in much lower number than (*Cit*⁻) *L. lactis* ssp. *lactis*.

In the washed butter (10th stage of production) the same microorganisms as in the previous stage were isolated, with a predominance of (*Cit*⁻) *L. lactis* ssp. *lactis*. The slight increase in the number of LAB (*Cit*⁻) was noticed (3.26 times in average). Doležalek cited by Stević (1962) identified that some strains of *L. lactis* ssp. *cremoris*, especially the (*Cit*⁻) *L. lactis* ssp. *lactis* can transform the part of the casein into amino acids, which are necessity for normal development of aromatic bacteria, including (*Cit*⁺) *L. lactis* ssp. *lactis*. Van Niel Kluyver and Derx cited by Stević (1962) detected diacetyl which is a product from LAB activity and in small quantities give the characteristic pleasant smell of cream and butter. This is confirmed also by Escamilla et al., (1996) who stated that diacetyl is an important component in the flavor of butter, although others, like acetoin, acetaldehyde and 2,3-butanediol play a role in the flavor, they are present in trace amounts. The mixture of all these compounds produced during the lactic acid fermentation in suitable proportions is what gives butter its characteristic flavor. Regarding this phase,

can be concluded that (*Cit*⁻) *L. lactis* ssp. *lactis* and (*Cit*⁺) *L. lactis* ssp. *lactis*, are important for the flavor development of butter, as a result of their metabolic activities.

In the buttermilk (11th stage) (*Cit*⁻) *L. lactis* ssp. *lactis*, *St.thermophilus*, (*Cit*⁺) *L. lactis* ssp. *lactis*, *Lb. delbrueckii* ssp. *bulgaricus* were isolated. In this stage the presence of (*Cit*⁻) *L. lactis* ssp. *lactis* was more dominant than other bacteria. In the buttermilk some increase in LAB compared with the number in milk after churning was noticed, where the average increase was 1.38 times

Results on total LAB in butter in our research were higher than the results on traditional butters reported by different authors (Idoui et al, 2009; Idoui et al., 2010; Kacem and Karam, 2006).

The distribution of LAB between butter and buttermilk is the following: in July buttermilk contains from 93.93% to 98.58% of the LAB and butter only 6.07% and 1.42%. In August 94.49% 95.93% and 5.51% and 4.07% while in September this percentage is 93% and 7% in buttermilk and butter accordingly. Similarly, in the cultured buttermilk Hammer (1948) defined the presence of (*Cit*⁻) *L. lactis* ssp. *lactis* in quite high numbers. The isolated microorganisms in the final phase probably participated in the creation a pleasant taste and flavour of buttermilk. This is in accordance with the statement by Sagdic et al. (2002) in his research on yayik butter where they concluded that only two organism starters, *Lb. delbrueckii* ssp. *bulgaricus* and *St. salivarius* ssp. *thermophilus*, were important for the favor of yayik butter.

Conclusions

Results obtained in this study showed that traditional butter and buttermilk contained a diversity of LAB. During production 128 isolates of cocci and rod-shaped lactic acid bacteria were isolated, 38 isolates from total were selected for determination. Lactic acid bacteria isolated from investigated products were determined as: (*Cit*⁻) *Lactococcus lactis* ssp. *lactis*, *Streptococcus salivarius* ssp. *thermophilus*, (*Cit*⁺) *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*, *Lactobacillus delbrueckii* ssp. *bulgaricus* и *Lactobacillus plantarum*.

These bacteria had a significant influence on the pleasant taste and flavor of butter and buttermilk.

The microbiological study of these local traditional products was not documented till now, and the results of this study provide an insight of the dynamic and composition of LAB in traditional butter and buttermilk. This study is expected to encourage more research on these products in Macedonia.

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DOG FIGHTING IN THE WORLD

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Abstract

Aim of this paper is to revise dog fighting in the World together Europe. Dog fighting, bear baiting, and bull baiting were officially outlawed in England by the Humane Act of 1835. Dog fighting, bear baiting, and bull baiting were also officially outlawed in whole European countries. Dog fighting has been illegal in Canada since 1892. Nowadays in USA dog fighting is illegal in all 50 states. Dog fighting is also illegal in most South American countries. In Afghanistan although dog fighting is banned by the Taliban it is still common throughout Afghanistan as a winter weekend pastime. In India dog fighting is not common, but can be found in some rural areas, and is illegal as defined by the Indian law. In Japan dog fighting seemed as legal. Dog fighting was considered a way for the Samurai to retain their aggressive edge during peaceful times. In Pakistan dog fighting and other forms of animal fighting are highly popular in all parts of the country even though it has recently been banned. It is deeply rooted in the rural culture. In Russia dog fighting is prohibited in much of the West. These blood sports were officially eliminated in 1835 as Britain began to introduce animal welfare laws. In Africa the Republic of South Africa is a country where one of the most dog fighting events happens. Dog fighting and the possession of any fighting equipment designed for dog fighting is illegal in Australia. "Restricted Breed Dogs" cannot be imported into Australia.

Key words: *Canis familiaris*, Europe, Pit Bull, blood sport, illegal sports, animal fight.

Introduction

Domestic livestock animals serve humanity by giving plenty of products including meat, milk, egg, honey, floss silk, wool, fur, skin, feather, intestine, work, amusement etc (Ertugrul et al., 1993). Moreover some animals have been and continue to be used for fighting by some mentally abnormal people. In various regions of the world there are popular animal fighting events including fighting of bull, camel, cock, cricket, dog, horse, partridge and ram (Köhne and Ewigleben, 2000). For centuries usually dog fighting has been principally enjoyed by men. Unfortunately, the popularity of this activity has increased (Anon, 2014a). There was little initial interference from the law, since it was possible to fight two dogs in any hollow or shed without attracting much attention, for

dogs fight with relatively little noise. They can easily be removed after the fight, carried away in sacks if their condition was likely to draw suspicion (Drable, 2014). For example in USA dog attacks and bites are not so common (Hussain, 2005). Although more than 20 fatalities happened each year in USA because of dog biting, there was no report that a man died during dog fight, but the dog. Maybe the 20 fatalities because of dog biting seem as tragic but at least 8.000 humans were killed by other human each year which was 200 times more than dog biting deaths (Lockwood, 1995).

Dog Fighting in America

Dog fighting has been illegal in Canada since 1892. The current animal cruelty sections of the Criminal Code of Canada offer greater protection for cattle and other working animals. It also gives less protection for wild or stray animals than for animals that are “kept for a lawful purpose” and make it extremely difficult to prosecute cases of neglect and dog fighting (Anon, 2014b).

Nowadays in USA dogfighting is illegal in all 50 states (Anon, 2014c) yet the dogfighting industry is booming in USA, concentrated in urban areas and the rural South (Villavicencio, 2007). Like any other illegal underground activity, it is too difficult to determine how many people may be involved in dog fighting. Estimates based on fight reports in underground dog fighting publications, and on animals entering shelters bearing evidence of fighting, suggest that the number of people involved in dog fighting in the U.S. is in the tens of thousands (Anon, 2014d). There are estimated to be about 40,000 people involved in professional dogfighting, an illegal blood sport with fight purses as high as \$100,000. Dog fighting has been reported in urban, suburban and rural settings in all regions of the country. Fighters were traditionally attracted to states with weaker penalties for dog fighting and animal cruelty, many in the American South—but laws continue to be made stronger throughout the country. As a result, this activity is no longer limited to any single area, but it is more likely to thrive wherever enforcement of anti-fighting laws is weak (Kalof and Taylor, 2007).

John Goodwin, an expert on animal fighting with the Humane Society, says there could be as many as 100,000 additional people involved in “street fighting” often involving young people in gangs. A database proved that reports of dog-fighting cases increased from 16 to 127 between 2000 and 2006. Dogfighting is a misdemeanor in Idaho and Wyoming, but a felony everywhere else. In some states where dogfighting is a felony, it is still perfectly legal to own a fighting dog or be a spectator at a dogfight. A bill signed by President Bush in May 2007, the Animal Fighting Prohibition Enforcement Act of 2007, Pub. L. 110-22 (Anon, 2015c), made the federal law against dogfighting tougher, by strengthening penalties, and banning interstate commerce and import and export related to animal fighting activities. Violators can now be sentenced to three years in jail and a \$250,000 fine. Previously the maximum sentence was a year in jail. In spite of the laws, dogfighting is big business in USA (Anon, 2014e).

Dog fighting is illegal in most South American countries yet it is widely practiced in much of Latin America, especially in Argentina, Peru and many parts of Brazil where dog fights are illegal. The American Pit Bull Terrier is by far the most common breed involved. The Fila Brasileiro and Dogo Argentino are also used as fighting dogs. The Dogo Cubano and Dogo Cordoba were used for fighting a century ago, but have become extinct (Anon, 2014e, f).

Dog Fighting in Asia

Despite being one of the poorest countries in the world, animal cruelty arguments don't carry much weight in Afghanistan, where dogfighting is a big business and a source of income for the owners of champion dogs. In Afghanistan a good fighting dog can be worth several thousand dollars. Although dog fighting is banned by the Taliban who outlawed the sport to prevent betting, which is not permitted in Islam, it is still common throughout Afghanistan as a winter weekend pastime. In Kabul, the capital city, the fights are public and policed for safety to the spectators. Dogs are not fought to the death, but to submission. Top dogs are worth as much as a new car (Villavicencio, 2007; Anon, 2014a; Motlag, 2014).

In India dog fighting is not common, but can be found in some rural areas, and is illegal as defined by the Indian law. It is also illegal to possess dogfighting materials such as videos, or to attend an event. In Japan dog fighting seemed as legal (Villavicencio, 2007; Ensminger, 2010; Anon, 2014e,g). Yozei, who became emperor in 877 at the age of ten, set dogs and monkeys to fight (Brinkley and Kikuchi, 1912). According to historical documents, Hojo Takatoki, the 14th shikken (shogun's regent) of the Kamakura shogunate was known to be obsessed with dog fighting, to the point where he allowed his samurai to pay taxes with dogs. During this period dog fighting was known as “inuawase”. Dog fighting was considered a way for the Samurai to retain their aggressive edge during peaceful times. Several daimyō, such as Chosokabe Motochika and Yamauchi Yodo, both from Tosa Province (present-day Kōchi Prefecture), were known to encourage dog fighting. Dog fighting was also popular in Akita Prefecture, which is the origin of the Akita breed. Dog fighting evolved in Kochi to a form that is called “token”. Under modern rules, dogs fight in a fenced ring until one of the dogs’ barks, yelps, or lose the will to fight. Owners are allowed to throw in the towel, and matches are stopped if a doctor judges it is too dangerous. Draws usually occur when both dogs will not fight or both dogs fight until the time limit. There are various other rules, including one that specifies that a dog will lose if it attempts to copulate. Champion dogs are called yokozuna, as in sumo. Dog fighting is not banned at a nationwide level, but the prefectures of Tokyo, Kanagawa, Fukui, Ishikawa, Toyama and Hokkaidō all ban the practice. Currently, most fighting dogs in Japan are of the Tosa breed which is native to Kochi (Anon, 2014e, g).

In Pakistan dog fighting and other forms of animal fighting are highly popular in all parts of the country. It is deeply rooted in the rural culture, where some 70 percent of the population of 167 million people lives. It has been a way for tribes, clans and the rural people to socialize while being entertained. Even though it has recently been banned by law, it is still being practiced in rural Pakistan, especially in provinces such as Punjab, Azad Kashmir, Sindh and Khyber Pakhtoonkhwa. There can apparently be as much as millions of rupees at stake for the owners of winning dogs, so different breeds have carefully been bred and selected specifically for the purpose, such as the Bully Kutta and Gull Dong (Longbottom, 2011; Nizami, 2012).

In Russia dogfighting is prohibited in much of the West. Even though animal cruelty laws exist, dog fighting is widely organized in some part of the country (Anon, 2015a). Throughout Central Asia and the Caucasus, and extending to the outskirts of Russia's capital, a form of the sport has thrived, cementing local legitimacy and gaining new followers since the Soviet Union's collapse 15 years ago. Animal rights advocates have long wished to have it banned in Russia and the rest of the former Soviet world, labeling it a cruel and a bloody diversion for gamblers and thugs. Laws prohibiting

dogfights have been passed in certain places, and in others dogfights are legally held generally using Caucasian Shepherd Dog, Georgian Shepherd, Central Asian Shepherd Dog, and Wolfhounds. Temperament tests, which are a common and relatively mild form of dog fighting used for breeding purposes, are fairly commonplace. Dog fighting is prohibited in Moscow by order of that city's mayor (Chivers, 2007; Villavicencio, 2007; Anon, 2014e).

Dog Fighting in Europe

These blood sports were officially eliminated in 1835 as Britain began to introduce animal welfare laws. Since dogfights were cheaper to organize and far easier to conceal from the law than bull or bear baits, blood sport proponents turned to pitting their dogs against each other instead. Dog fighting was used as both a blood sport (often involving gambling) and a way to continue to test the quality of their stock. For decades afterward, dog fighting clandestinely took place in pockets of working-class Britain and America. In recent years the inner cities, especially in London have seen a steady rise in the number of convictions for dog fighting, primarily among teenage youths of Pakistani descent (Anon, 2014e). A research study was carried out to discuss financial aspects of illegal dog-fighting in the UK and to reflect upon and discuss the difficulties of researching illegal entrepreneurial activities such as dog-fighting which were operated for criminal profit. According to the study dog fighting activities were conducted by urban criminals often in a rural setting. Those crimes invariably occurred in a closed social environment to which the authorities and the academic researcher cannot legitimately gain access. In the case study the illegal activities can legitimately be regarded as being an entrepreneurial activity as they entail trading in a Kirznerian sense as well as financial implications associated with gambling (Smith, 2011).

Although the dog fighting is illegal in the EU Member States, but fights are frequently being organized throughout Europe and the EU, in which dogs are literally forced to fight for their lives. The organized dogfights operations is very extensive and widespread i Europe (EU), more than the general public and the police in Europe are aware of. The dogs are born, live and die as a tool in a world of cruel violent people, money and prestige. This is one of the most serious cruelty to animals that exists in the world, and in Europe. Nevertheless, this last in silence without any direct attention by media or the society. The dogs live many times in terrible conditions and lacking affection and love. They suffer all their lives and always die in pain - thousands of dogs have to suffer every day, all year round. It is all about breeding, betting and gambling, which are advertised in a highly sophisticated and organized way via the internet. The most used breeds are dogs with already enhanced characteristics and by selective breeding, people are trying to increase the dogs' qualities and to improve the dogs' characteristics, like low stimulus threshold, aggression, tenacity and combativeness. Dogs used in this cruel sport are usually Pitbull, Rottweiler, mixed breeds but also other purebreeds. The dogs are not born vicious or aggressive, but the unscrupulous individuals begins to train the dog when it's puppy and triggers the dog to want to fight and bite, which is easier for some breeds to embrace. All dogs in this industry that do not classify themselves in this cruel sport, are brutally killed. Everything is done illegally, which means that those dogs are never insured or receive regular veterinary care This industry has its own vets, medicines and it is common for the owners themselves to perform surgery on injured dogs. Many dogs who are not used in actual fight are used as training sacrifice (bait dog or target dog). The champion dogs can

practice their tactics on the "bait" who usually has the mouth taped in order to only serve as a training body and is killed by being chewed to death without being able to defend itself. Many stray dogs and cats are used and owned companion animals are stolen, but also dogs who are exclusively bred by these insensitive people are used for this cruel practice. Street-fighting dogs treated for stab wounds - Animal health workers are treating dogs for stab wounds, cigarettes burns and broken bones as reports of street fighting soar. Two-thirds of phone calls about dogs fighting are linked to young people who wield them as weapons. The animal charity said the number of reports of dog fighting has increased tenfold from 2004 when there were 24 reports to 284 last year. Of the reports received last year, 188 related to "macho" dogs such as bull terrier-type breeds and Rottweilers. David Grant, of the RSPCA's Harmsworth Animal Hospital, north London, said the capital is the nation's hotspot for dog fighting. But he said colleagues face young people who use dogs to fight and intimidate in Merseyside, West Yorkshire, and the West Midlands. Mr Grant said: "My staff are treating more dogs than ever for fight wounds and injuries sustained as a result of them being kept as status symbols by young people who think having a dog makes them look tough. "I've had to treat dogs with stab wounds, broken bones and even cigarette burns to their heads. The torture some of these poor animals have suffered is simply horrific." (Grant 2015)

Dog Fighting in Africa

In Africa the Republic of South Africa is a country where one of the most dog fighting events happens. For example a report stated the Animal Welfare Society was called out at least three times a week to dog fights in the area of Cloetesville, Stellenbosch. In the country the American Pit Bull Terrier is the most often used dog in dog fights. It is known that dog fighting has been declared illegal in the Republic of South Africa. On the other hand dog fighting is still very popular in the underground world. The National Council of Society for the Prevention of Cruelty to Animals is the largest animal welfare organization in Africa. It has been the organization that has conducted the most raids and busts. For example 18 people were arrested, and 14 dogs were involved in 2013. Dog fighting is mostly practiced in the Western Cape, in the townships area where gangs and drugs are mostly associated with dog fighting. Dog fighting has been documented in South Africa, particularly in the Western Cape region of Stellenbosch. The Stellenbosch Animal Welfare Society frequently responds to complaints of night-time dog fighting in the town of Cloetesville in which hundreds of dogs fight. Young children may be used to transport fighting dogs to avoid arrest of the owners (Anon, 2014e, h, i).

Dog Fighting in Australia

Dog fighting and the possession of any fighting equipment designed for dog fighting is illegal in Australia. Dog fighting in Australia is often associated with gambling activities and other illegal practices such as drug dealing and firearms. The RSPCA is concerned that dog fighting involves the suffering or even the death of dogs for the purpose of entertainment. The illegal nature of dogfighting in Australia means that injured dogs rarely get veterinary treatment placing the dog's health and welfare at even greater risk. "Restricted Breed Dogs" cannot be imported into Australia. These include the Dogo Argentino, the Japanese Tosa, the Fila Brasileiro, the Perro de Presa Canario and the American Pit Bull Terrier. Of these, the Pit Bull Terrier and the Perro de Presa Canario are

the only breeds currently known to exist in Australia and there are strict regulations on keeping these breeds, including a prohibition on transferring ownership (Anon,2014e, h,j).

Conclusion

It is too difficult to understand that some people defend dog fighting. According to those abnormal people dog fights are necessary and should be carried out (Gasimzadeh, 2014). This kind of people is rare in community but they are always present in the community and always will be. The most important thing to prevent dog fighting is inform related police or gendarme forces. Also penalties should be more increased.

Acknowledgements

I would like to thank John Ensminger and Marc Paulhus for their constructive comments, careful scientific revision and English edit.

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SAWDUST-ROUGHAGE SOURCE IN THE FEED MIXTURES FOR FATTENING STEERS

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Abstract

The influence of sawdust as a roughage source was evaluated in the feeding of 42 holstein fattening steers. The substitution of 7% compound feed, based on corn soybean meal and sunflower meal, with 7% of sawdust, influenced on decreased gain for 0.12%, decreased consumption for 0.84% and decreased feed conversion(NEGF-MJ/kg) for 0.50% in relation with control group. Statistical data of final weights, consumption and feed ratio of fattening steers, showed that sawdust substitution in the rations for fattening steers didn't show negative effects on productive results, i.e., the differences between groups were not statistically different (confidence interval 99%).

Key words: sawdust, corn, soybean, sunflower, daily gain, consumption, conversion, fattening steers, productive results.

Introduction

Feed as a non-genetic factor played significant role in normal exploitation of domestic animal's genetic performances. Modern science has strongly involved in finding new techniques and technologies, by using appropriate ways and methods for better and cheaper animal production, without avoiding of the ecology, as a priority.

Feed cost is major input in productive cost of animal products. It has range from 30% (at the young categories) to 90% (at the older and non-productive) from all inputs at the ruminants, in dependence of the type of the feed and its way of using.

The nutrition of ruminants is predominantly based on using of more than 50% roughage feed in the daily ration, in dependence of physiological status and the intensity of programmed production. The ruminants are excellent converters of roughage feed into the good animal product. The cattle rations are with different concentration of bulkiness-roughness, which is coming from the crude fiber of the feeds. The cellulose, hemicellulose and lignin are major part of the fraction crude fiber. It influenced in most cases negatively. However, the certain percentage of crude fiber could be tolerated, because it provides long-term satiety and enables larger cellulolytic population and enzymatic activity in the stomach medium. The roughage source in the rations of ruminants was subject in many previous researches, which showed various effects at the fattening ruminants.

The literature confirmed that the enzymes of cellulolytic microbes, which are existing in the digestive tract of the domestic animals, are capable to degrade the crude fiber and its components (cellulose, hemicellulose and lignin).

The most common cellulolytic microbes are bacteria and protozoa. Also, it was confirmed that the cellulase secretion was evidenced at the lower animal species, like spiders and other arthropod, but its cellulolytic activity is not enough to degrade the crystal form of cellulose. The capability of termite to degrade the lignin as good as the cellulase is connected with presence of aerobic fungus in the tunnels of its structure.

Because of the above mentioned facts, it scientifically justified, and also important for the practise to estimate the influence of substitution of energy feeds with a by-products, like sawdust, as a bulkness source in the mixtures for fattening steers on its productive results.

The literature data showed various final effects. The different types of feeds, as corn cob (Guerin et al., 1955) cotton hulls (Conrad et al., 1967), rice hulls (Tillman et al., 1969), oyster shell (Perry et al., 1968), were used in high energy rations for ruminants with various grades of success.

Using of straw and wooden by-products (sawdust, pulpwood etc) as a energy source was researched in many cases. During the World War Two, Scandinavian countries used more the 1.5 million tons of sulfate and sulfite pulpwood from different conifer wood for feeding of cattle and horses, due to a lack of animal feed (Hvidsten and Homb, 1951; Nordfeldt, 1947).

Wooden by-products as sawdust evoked attention as a possible roughage substitutes. Encouraging results were obtained when sawdust were included in the final feed mixtures for fattening steers (Anthony et al., 1969) and in the high concentrated mixtures for dairy cows (Cody et al., 1968). Some other reports (Kinsman et al., 1969, Dinius and Oltjen, 1971) indicated satisfactory results with used sawdust and newsprint in the rations for fattening steers. The aim of the researches of El-Sabban et al. (1971) was to verify the effect of sawdust as a roughage substitute in the final rations for fattening steers. Their researches were performed on Hereford fattening steers. The rate of inclusion of the sawdust in the two groups was 5 and 15%, and for the third group roughage source was grass hay (Timothy hay), with inclusion of 5%. The obtained results showed satisfactory effects, up to 15% rate of inclusion of sawdust, with the little advantage for the coarser fractions of sawdust.

Material and methods

The experiment was performed on the cattle farm in the Skopje region. The experiment was based on fattening steers, which were included in the experiment on the age of 130-140 days and 150-160 kg live weight. All animals were from holstein friesian breed. The animals were divided into two groups with the same number per each group. The feeding and watering of animals was ad libitum.

The trial included 32 animals divided on two groups. The duration of the experiment was 267 days, divided on two periods: preliminary which lasted 15 days and the experimental, which lasted 252 days. The experimental period was divided on two phases: first, which lasted 84 days, respectively to the body live weight of 250-260 kg, and the second, which duration was 168 days, respectively to the final body live weight of 450 kg. The body live weight measurement was performed periodically. Also, the feed

consumption was measured individually per each animal. The nutrient content of the feed mixtures and hay was analyzed twice per the experiment. The fattening steers were fed with appropriate feed mixtures. Two feed mixtures were included. First feed mixture was prepared for the first phase (with duration of 84 days) according to the feed role book for steers with weight to 250 kg, and the second mixture was prepared for the second phase, for the the steers above 250 kg body live weight (duration of 168 days).

The aim of the research was to investigate the influence of energy feed substitution with the sawdust, as a source of a bulkiness in the feed mixtures for fattening steers, and justification for using of different byproducts into the rations of fattening steers.

The steers from the control group were fed with feed mixtures without sawdust, and the experimental steers were fed with inclusion of sawdust. The substitution was 7 % of corn with 7 % of sawdust. The sawdust was from oak, polar and fir.

Table 1. Composition and nutritive value of mixture for nutrition of steers in the first phase of fattening (150-250 kg)

Feeds in %	Frst phase of fattening (150-250 kg)	
	K (I)	E (II)
Maize	73.60	66.60
Sawdust	/	7.00
Straw	/	/
Soya bean meal	12.00	12.00
Sunflower meal	10.00	10.00
Limestone	1.00	1.00
DCaP	1.40	1.40
Salt	1.00	1.00
Premix	1.00	1.00
Total	100.00	100.00
Crude protein, %	14.97	14.65
Oat units/kg	1.25	1.17
NEGF, MJ/kg/DM	6.88	6.27

Table 2. Composition and nutritive value of mixture for nutrition of steers in second phase of fattening (250-450 kg)

Feeds in %	Second phase of fattening (250-450 kg)	
	K-I	E-II
Maize	80.60	73.60
By product of three	/	7.00
Starch	/	/
Soya bean meal	/	/
Sunflower meal	15.00	15.00
Limestone	1.00	1.00
DCaP	1.40	1.40
Salt	1.00	1.00
Premix	1.00	1.00
Total	100.00	100.00
Crude protein, %	12.76	12.35
Oat units/kg	1.25	1.17
NEGF, MJ/kg/DM	6.70	6.01

The feed test samples were taken on the equal time periods. The procedures were based on the ISO standards for sampling, preparation and testing of animal feeding stuffs.

The control measuring was performed on the calibrated technical balance. The average live weight was calculated on the base of the weight measurement. The total gain was calculated from the difference of the live weights. The daily gain was calculated from the duration of the certain phase, and for the whole period.

The consumption of the feed mixtures was measured per animal, per group, per phase and for whole period, on the base of the daily consumption. The feed ratio was calculated from the consumption and live weights, per phase and for the whole period.

The data were statistically processed.

Results and discussion

The data for the average body live weight are showed int the Table 3.

Table 3. Body weight of fattening steers in the experiment, [kg]

Parameters	Groups	
	K-I	O-II
Body mass of the beginning of trial, kg	154.12	154.75
Age of the beginning of trial, days	130-140	130-140
Body mass of the end of the first phase of trial, kg	257.37	252.20
Days of fattening	84	84
Body mass of the end of the second phase of trial, kg	452.25	452.51
Days of fattening	168	168

The animals at both groups had equal body live weights, and there was no significant difference between the groups (Table 3). The body live weight of control fattening steers in the first fattening phase (150-250 kg) was 257.37 kg, and 252.20 kg at the experimental fattening steers. The body mass of experimental fattening steers in the second phase of the fattening (250-450 kg) was 452.51 kg, and at the control steers 452.25 kg. The dynamics of body live weights for the whole fattening period is showed in the Graph 1.

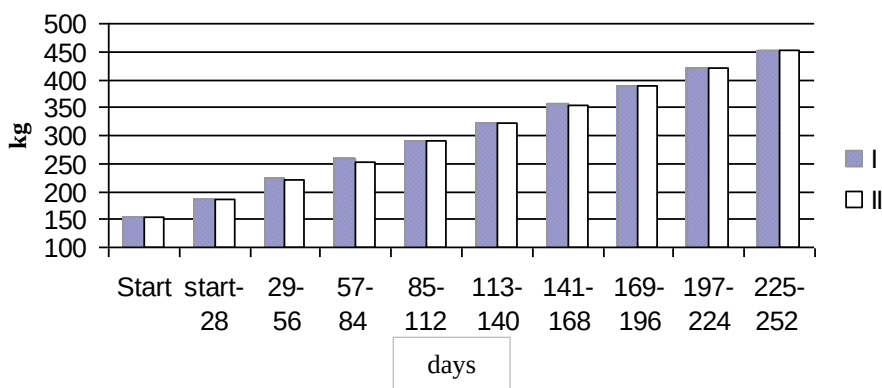


Figure 1. Live weight dynamics per fattening periods [kg]

The total and daily gains of two groups per fattening phases are presented in the Table 4.

Table 4. Total and daily gain of fattening steers on the phase in the experiment, [kg]

Parameters	Groups	
	K-I	E-II
Average total gain for first period of fattening (84 days)	103.25	97.75
Average total gain for second period of fattening (168 days)	194.87	200.01
Average total gain for all period of fattening (252 days)	298.12	297.76
Average total gain for first period of fattening (84 days)	1.229	1.163
Average total gain for second period of fattening (168 days)	1.16	1.191
Average total gain for all period of fattening (252 days)	1.183	1.182

The average daily gains of groups showed difference (Table 4). Higher daily gain was measured in the first phase at the control group (5.68 %). The average daily gain in the second phase was higher at the experimental group (2.68 %). The average daily gain at the both groups for the whole period was without significant difference ($p < 0,05$).

The dynamics of the total and daily gains per months are presented in the Graph 2.

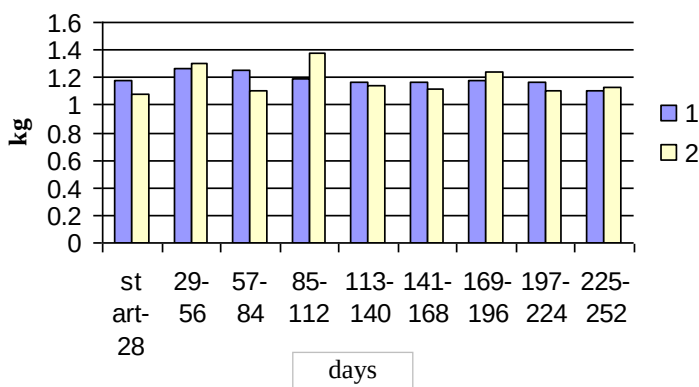


Figure 2. Daily gain dynamics (per months)

The average daily feed consumption is showed in the Table 5.

Table 5. Daily feed and nutrient consumption in the experiment, [kg/day]

Parameters	Control group	Exp. group
- Feed mixture	6.07	6.75
- Alfalfa hay	1.00	1.00
Dry matter, total	6.19	6.84
Crude protein, total	0.959	1.026
Oat units, total	8.13	8.18
NEgf, MJ/kg	44.70	44.78

The total consumption of alfalfa hay feed for the whole fattening period per head was 252 kg. The average daily feed mixture consumption for whole period per head was 6.07 kg (I) and 6.75 kg for the second group.

The average daily crude protein consumption was at first group 0.959 kg, and 1.026 kg at the second group.

Average consumption of oat unit and NEgf per day/per head was 8.13 and 44.70 MJ at the control, and 8.18 and 44.78 MJ/kg at the experimental group.

The Table 6 contents data for the total and daily feed and energy consumption per fattening periods-phases

Table 6 Daily feed and nutrient consumption per fattening periods, [kg/day]

Parameters	Period	Control group	Exp. group
Feed	1	4.08	4.71
- Feed mixture	2	7.06	7.77
- Alfalfa mea	1	1.00	1.00
	2	1.00	1.00
Dry matter, total	1	4.44	5.02
	2	7.07	7.74
Crude protein, total	1	0.766	0.832
	2	1.056	1.101
Oat units, total	1	5.65	5.87
	2	9.37	9.33
NEgf, MJ/kg	1	31.89	33.33
	2	51.10	50.50

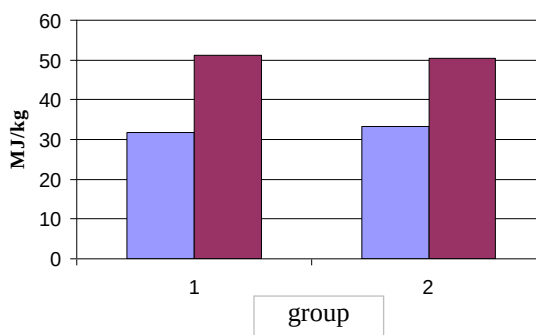


Figure 3. Net energy consumption (MJ/kg) per fattening periods

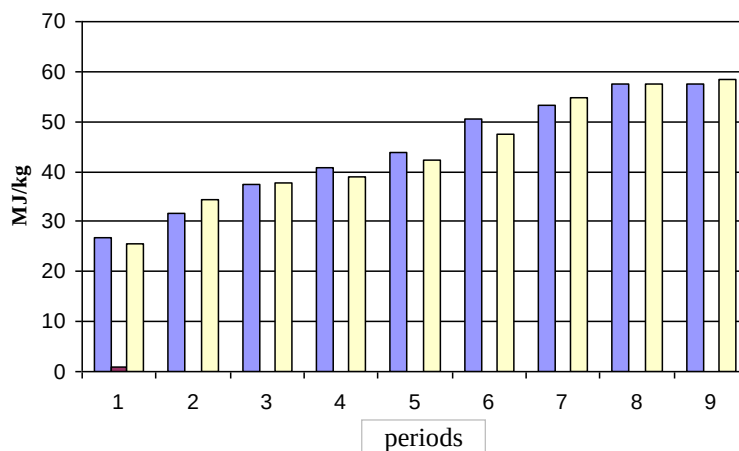


Figure 4. Net energy consumption dynamics (MJ/kg) in the experimental period
Feed consumption and feed ratio is presented in the Table 7.

Table 7. Feed and nutrient ratio per fattening periods, [kg]

Parameters	Control group	Exp. group
First fattening phase (150-250 kg)		
- Feed mixture, kg	3.32	4.05
- Alfalfa hay, kg	0.82	0.84
- Dry matter, kg	3.61	4.31
- Crude protein, kg	0.623	0.715
- Oat units/kg	4.60	5.20
- NEgf MJ/kg	25.97	28.58
Second fattening phase (250-450 kg)		
- Feed mixture, kg	6.09	6.52
- Alfalfa hay, kg	0.85	0.85
- Dry matter, kg	6.09	6.53
- Crude protein, kg	0.910	0.928
- Oat units/kg	8.08	7.26
- NEgf MJ/kg	44.05	42.42
Whole period (150-450 kg)		
- Feed mixture, kg	5.13	5.71
- Alfalfa hay, kg	0.84	0.85
- Dry matter, kg	5.23	5.80
- Crude protein, kg	0.811	0.870
- Oat units/kg	6.87	6.52
- NEgf MJ/kg	37.87	38.06

The data in the Table 7 showed relatively equal situation at the both groups. The feed ratio for the whole period was 5.13 kg/kg for the first group, and 5.71 kg/kg per second group. The energy ratio for the second group was 6.52 oat units/kg gain, or 38.06 MJ/kg gain, and for the first was 6.87 o.u/kg, or 37.87 MJ/kg gain.

Conclusion

On the bases of obtained results from the experiment it could be concluded that:

- Substitution of the 7 % of standard compound feed, based on the corn, soybean meal and sunflower meal, with 7 % of sawdust influenced on:
- 0.12 % lower gain,
- 0.84 % lower consumption and
- 0.74 % better feed ratio (NEGF-MJ/kg)

In comparison with the control group.

The processed results from the final body live weights, consumption and feed ratio of fattening steers showed that the substitution with the sawdust didn't influence negatively on the productive results, respectively the differences between the groups were without significance ($p < 0.05$).

The partial substitution of corn with the sawdust in the rate of 7 %, hadn't negative effects on the productive results at the fattening steers.

In general, applied treatments didn't influence negatively on productive results and on the health status of fattening steers, which allow real possibility for partial substitution of corn with the sawdust.

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COMPARISON OF AGRICULTURAL POLICY IN CIS COUNTRIES – first assessment

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Abstract

The goal of the research was to assess the agricultural policies of 8 CIS countries that are of interest due to the changes in the geopolitical situation following the dissolution of the Soviet Union, as well as more recent developments in the region. They hold a great production potential and are relatively unanalysed from the point of view of agricultural policy. We have attempted to analyse their agricultural policies and the effects thereof on production and trade. The main research question included: the agricultural policy focus (challenges and objectives), key instruments used, evaluation of market price support, systematisation and quantification of budgetary transfers and potential impact of agri-policy on trade and production. Analysis was conducted both qualitatively (through document analysis and literature review) and quantitatively (using the OECD PSE methodology of policy analysis). The key issues in the region are food security and competitiveness, while policy approaches range from strong interventionism to almost complete liberalisation. Tax and export policy play a prominent role in most countries, while budgetary support is relatively low (approximately 1/3) compared to EU and OECD averages; transfers to producers dominate (especially input subsidies and on-farm investment support) in all countries, while support to general services is weak. While prices for crops are near world prices, prices for animal products are fairly high in some countries, pointing to high developmental needs. It is possible to discern four rough clusters of countries.

Key words: CIS, agricultural policy, trade, PSE, producer support

Introduction

Since the early 1990s, CIS 8 countries (Armenia, Azerbaijan, Belarus, Georgia, Kazakhstan, Moldova, Russia and Ukraine) have undergone transition from a centrally planned to a market-oriented economy. In the context of agricultural production and trade, economic and institutional reform meant the abolishment of central controls and planning, privatisation of production assets and land reform, reductions in government intervention in internal markets, price and trade liberalization and economic stabilization (Lerman *et al.*, 2004; Csaki and Forgacs, 2008; Buchenrieder *et al.*, 2009; Lerman, 2009). There is a lack of recent comprehensive analyses dealing with the changes in agricultural policy and their impacts on production and trade. The state of agriculture and agricultural policy is

monitored by the OECD for Russia, Ukraine and Kazakhstan (in the frame of the Producer Support Estimate – PSE calculations – see OECD, 2013, and OECD, 2015a). Many studies and analyses are also published by the FAO (e.g. country-specific agroindustry briefs; see FAO, 2013b-i), but there is no complete systematisation and quantification of budgetary transfers and market support to agriculture for countries that are not observed by the OECD. Using available data from the literature and data collected in the framework of the AGRICISTRade project¹, we have attempted to systematise and qualify agricultural budgetary transfers and assess the effect of policy on producer price levels in order to present and compare agricultural policy and some of its impacts on prices, production and trade.

The main research questions included: the agricultural policy focus (challenges and objectives), key instruments used, evaluation of market price support, systematisation and quantification of budgetary transfers and potential impact of agri-policy on trade and production.

Material and methods

We analysed primary sources of information: national strategies for agricultural policy. We also made much use of the country reports prepared by experts in the framework of the project, as well as direct consultation with them. We supplemented this information with country-specific research and expert papers. These include OECD (e.g. the Agricultural Policy Monitoring and Evaluation Report (OECD, 2013)) and FAO documents (FAO, 2013a), reports prepared by FAO for the EU in the framework of the European neighbourhood policy mechanism, and USDA's reports (e.g. USDA, 2012). Finally, we analysed the conceptual framework of national agricultural policy measures, relying most on the country reports prepared by country experts, and supplemented this with reports and policy analyses issued by various international organisations (OECD, FAO), as well as some scientific papers.

Systemization and qualification of policy instruments and measures is generally based on the OECD PSE/CSE approach to policy analysis (OECD, 2015a). Given that for five out of eight countries not all data needed for the calculation of support indicators according to the OECD methodology (OECD, 2015b) are available, support to agriculture was assessed using simplified measurements for all countries using the Nominal Protection Coefficients (NPC) with the following formula:

$$\%NPC_i = \frac{PP_i}{RP_i} * 100 - 100$$

(1).

The quantitative assessment of price protection is based on the calculation of the percentage ratio between the price received by farmers and the border price for the selected set of commodities. The NPCs by country were calculated only for commodities which represent at least 1% of the total value of production (VP). For Russia, Ukraine and

¹<http://www.agricistrade.eu/document-library>

Kazakhstan, NPCs were calculated using the countries' own reference prices as assessed by the OECD; for Armenia, Azerbaijan, Belarus, Georgia and Moldova, data on own trade prices are not available, so Russian and EU reference prices were used.

Budgetary support was analysed by group of measures respecting the basic OECD PSE/CSE classification scheme (budgetary transfers to producers, general services and consumers) (see OECD, 2010) and compared using relative indicators. The basic relative indicator used for comparison of the level of support was the value of transfers related to the value of agricultural production. For Kazakhstan, Russia and Ukraine, the primary source of data was the OECD PSE/CSE database. For Armenia, Azerbaijan, Belarus, Georgia and Moldova, the basic data were taken from the consolidated databases by country, established as a part of the AGRICISTRADO project (AGRICISTRADO database). Datasets cover different time periods and are not complete in terms of measures covered for all countries.

Results and discussion

Agricultural policy framework

For CIS 8, agriculture still represents an important percentage of both the GDP and employment and is an important labour sink (Swinnen *et al.*, 2010; Lerman, 2004) and social safety net. As a consequence, all the countries have strategic policy documents related to the development of agriculture. They generally have no fixed structure that follows the logic problems-objectives-instruments-financial frame-impacts, but stop at the description of issues, general goals and perhaps mention some policies and instruments. Specific budgetary plans, instruments and impact assessments are rare, also due to the fact that often there are no funds available for the practical implementation of these strategies.

The stated priorities are quite similar, with a strong production-oriented character emphasising food security. There are also other priorities, indicating the primarily developmental role of agricultural policy (see e.g. Norton *et al.*, 2014): competitiveness, productivity, efficiency, farm investment, export orientation, food value chains (cf. OECD, 2011; FAO, 2013a-i). Some concepts that have been gaining momentum in the EU, like environmental concerns, risk management, knowledge generation and transfer, are marginal.

Market-price instruments range from heavily controlled agricultural markets (BY), to almost complete market deregulation, mostly in the smaller countries (AM, AZ, GE, MD). Russia is somewhere in the middle, while Ukraine and Kazakhstan are closer to the smaller countries. Import protection is present in all the countries and depends on political economy, regional conflicts, and trade liberalisation. The Eurasian Customs Union, as well as Free Trade Agreements, the WTO and various trade agreements with the EU², play important roles. The level of import protection ranges from low or modest, mostly in the form of non-tariff barriers (e.g. AM, MD), to heavy-duty tariffs and tariff quotas (RU, BY, KZ), in certain cases even bans, culminating in the 2014 Russian food embargo. While some countries are attempting to enhance export (e.g. AM and to a lesser extent MD),

² Partnership and Cooperation Agreements with Armenia, Azerbaijan, Kazakhstan and Russia; Association Agreements with Georgia, Moldova and Ukraine; Trade and Cooperation Agreement with Belarus; see <http://ec.europa.eu/trade/policy/countries-and-regions/>.

others are working primarily on domestic food security, closing their borders (using various bans, grants or permits) or promoting export only in instances of large surpluses (e.g. RU). Almost all the countries have in place some kind of tax policy favouring agriculture, e.g. exemptions from land and profit taxes, as well as special VAT regimes. In Ukraine the lion's share of budgetary transfers to producers is done via the system of taxation.

Price support

In 2011-2012, prices received by farmers were generally above world price levels in Kazakhstan, Armenia, Azerbaijan, and Georgia. A lower price level can be found in Belarus, Moldova and Ukraine; Russia's prices are on average close to reference levels. In Belarus, Moldova, Russia and Ukraine, overall %NPC was negative, mainly due to highly negative overall protection rates for crops. Although in all countries, %NPC was negative for almost all representative crop commodities, overall %NPC for crops was influenced by highly negative protection rates for potatoes, which represent a high share of the total VP. If the %NPC for potatoes is set at zero, the estimated overall %NPC for crops is considerably less negative, particularly in Ukraine, Belarus, and Russia, resulting in a less negative total protection rate in Ukraine and Belarus and a modest positive aggregate %NPC in Russia.

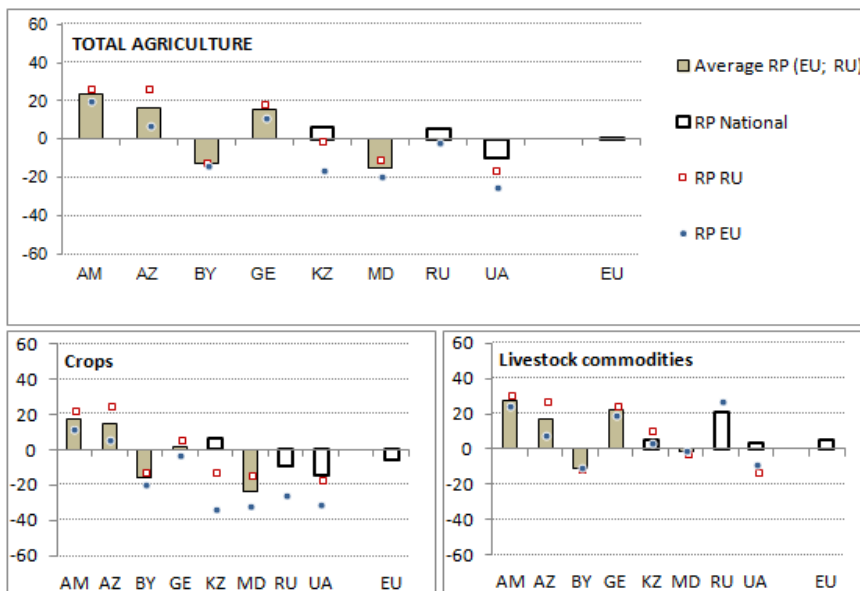


Figure 1. CIS 8: Aggregate percentage ratio between the producer price and reference border price (%NPC) by country, 2011-2012 average

Source: Own calculation based on AGRICISTRAD database and OECD PSE/CSE database

Throughout the region, livestock production enjoys a higher level of price protection than crops. For livestock commodities, domestic prices were higher than or close to the reference price levels in all observed countries, except in Belarus, where protection rates for livestock commodities were also generally negative. For most countries the most

SECTION 2. AGRICULTURAL ECONOMICS

protected products are pig meat and poultry, while the least protected are potatoes, beef and sheep meat, as well as wheat and maize.

Table 1. Overview of the relative position of producer prices in relation to reference prices for selected representative commodities by country, 2011-2012

Producer prices	AM	AZ	BY	GE	KZ	MD	RU	UK
Higher (NPC 10% or more)	Wheat Barley Beef Pig meat Poultry Eggs	Wheat Barley Potatoes Poultry Milk Eggs	Pig meat	Wheat Barley Beef Pig meat Poultry Eggs	Wheat Sunflower Beef Pig meat	Pig meat Poultry	Beef Pig meat Poultry Milk	Sugar Poultry
Rather aligned (NPC $\pm 10\%$)	Sheep meat Milk	Maize	Sugar Beef Poultry	Sunflower Milk Maize	Potatoes Poultry Sheep meat Milk Eggs	Sugar Sunflower	Sunflower	Pig meat Eggs
Lower (NPC - 10% or less)	Potatoes	Beef Sheep meat	Wheat Maize Barley Potatoes Milk Eggs	Potatoes Sheep meat	Maize Barley	Wheat Maize Potatoes Beef Sheep meat Milk Eggs	Wheat Maize Barley Sugar Potatoes Eggs	Wheat Maize Barley Potatoes Sunflower Beef Milk

Source: own calculations

Budgetary transfers

The relative level of budgetary support to agriculture in the CIS 8 region varies considerably. In 2011-2012, it ranged from around 1% of the total value of agricultural production in Armenia and 3% in Moldova, to almost 15% in Belarus. In other countries the proportion of budgetary support in the total value of production is between 6% and 11%. In the EU, budgetary transfers to agriculture amounted to around 22% of the total value of production in 2011-2012.

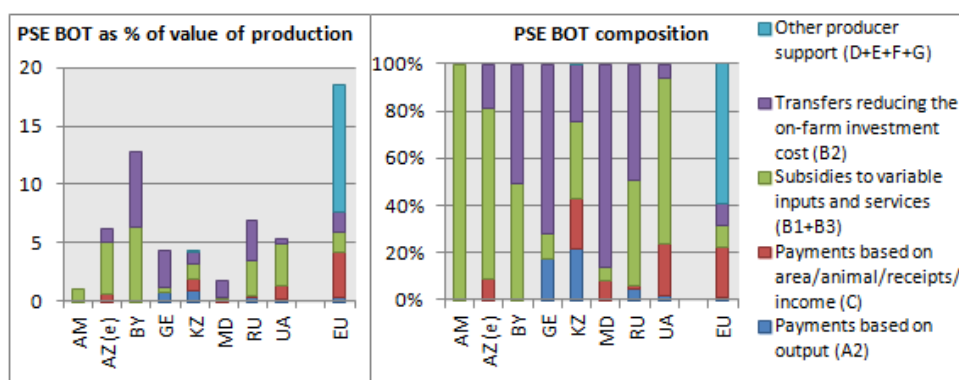


Figure 2. Budgetary transfers to producers (PSE BOT) by main categories

Source: Own calculation based on Policy database (AM, AZ, BY, GE, MD), OECD/PSE database (KZ, RU, UA, EU)

Transfers to producers individually dominate in all countries in the region, as well as in the EU, with shares in overall budgetary transfers to agriculture ranging from 56% in Kazakhstan to 87% in Belarus (85% in the EU). Support to general services for agriculture accounts for between 20% and 40% of the total budgetary transfers in most countries; only in Belarus is this share smaller (12%) and below the EU average (14% in 2011-2012). Budgetary support to consumers generally has the smallest share in total budgetary support and is non-existent in Armenia, Moldova and Ukraine; in other countries its contribution to total budgetary support to agriculture accounted for 1% (BY) to 7% (RU) (1% in EU).

The relative level of budgetary support to producers is by far the highest in Belarus (13% VP), followed by Russia (7%), Azerbaijan (6%), Ukraine (5%), and Georgia and Kazakhstan (4%). In Armenia and Moldova it is considerably lower (around 1% and 2% of VP, respectively). Only in Belarus is it comparable with the EU (almost 19% of the VP on average), while other countries are far behind. In all countries in the region, budgetary transfers to producers are provided exclusively in production-coupled forms of support. In the EU, coupled support accounts for only about 40%, largely due to the gradual replacing with less trade-distorting forms. Input subsidies are the largest producer support instrument in all the countries. In Armenia, Moldova and Belarus, this is generally the only form of budgetary support to producers; in Russia its contribution is close to 95%. In Azerbaijan, the share of input subsidies in total budgetary support to producers is estimated to be around 90%. In Georgia and Ukraine, it is around 80%. Subsidies to variable and fixed inputs are the largest support item in Kazakhstan, as well, but with a smaller contribution to the total budgetary transfers to producers (below 60%) compared with the other countries. In Kazakhstan, more than 40% of support is granted in the form of direct payments. These are also quite important in Ukraine, Georgia and Azerbaijan. In Russia, direct payments are less significant and are predominantly output payments linked to livestock products.

In most countries, the relative level of support to general services for agriculture accounts for 1% to 3% of the total value of agricultural production (around 3% in the EU). The level of general support is marginal in Armenia. In Kazakhstan, Moldova and Armenia, budgetary support to public services, institutions and infrastructure is directed predominantly for inspection and control, in Georgia for infrastructure and in Ukraine and Russia for knowledge generation. In Belarus and Russia, quite a large part of general support is non-specified (miscellaneous).

Estimation of total transfers to producers

Taking into account both aspects of policies, market measures and budgetary support, it can be concluded that in the most recent years for which the data for all observed countries are available (2011-2012), agricultural producers were generally supported in Armenia, Azerbaijan, Georgia, Kazakhstan and Russia, and taxed in Moldova and Ukraine. In Belarus, overall total support to producers seems to be close to zero.

The aggregate level of support to producers was predominantly influenced by (positive or negative) market price transfers³, even though budgetary support also played

³ Market price transfers were calculated as the price gap between domestic and reference price (MPD) in relation to the value of production (by commodity and for all selected representative commodities in a country). MPD for potatoes was set at zero for all countries.

quite an important role in most countries. In Russia, budgetary transfers to producers contributed about half of the overall level of support, while in Belarus the relatively high budgetary support more or less compensated for the overall negative price transfers.

The relative level of total transfers to producers is close to the EU average only in Armenia, Azerbaijan and Georgia, countries for which the estimated price support is the most uncertain due to unreliable data on domestic prices and also the effect of overvalued exchange rates (Armenia); in other countries in the region, support to producers is below the EU level. In the EU, almost all producer support is provided through budgetary transfers, as the average level of domestic prices was almost aligned to border price levels in 2011-2012 (OECD, 2015).

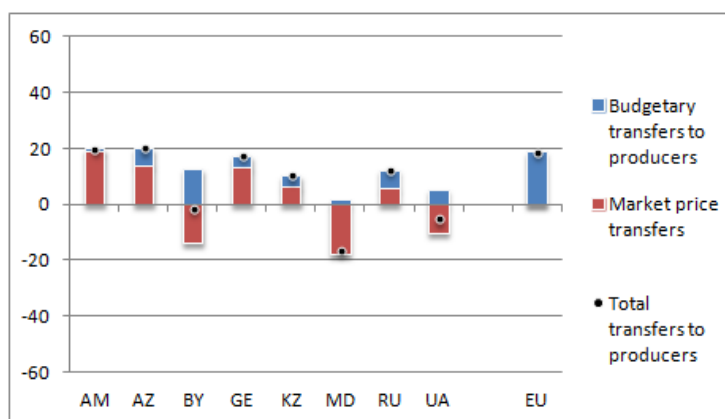


Figure 2. Estimated level and composition of total transfers to producers (in % of the total value of agricultural production), 2011-2012 average

Source: Own calculation based on Statistics and Policy database (AM, AZ, BY, GE, MD), OECD/PSE database (KZ, RU, UA, EU)

Conclusions

By calculating NPCs and budgetary transfers, we have acquired an aggregate evaluation of total transfers to producers, expressed as a percentage of value of production. The evaluation of producer support also allows for clustering of countries according to the nature of agricultural policy:

- Transcaucasus countries (AM, AZ, GE) – the rate of support is about 20% of the value of agricultural production and comparable as a percentage with the European Union, but differs significantly in composition.

- Russia and Kazakhstan. Support is at the level of 10% of the value of agricultural production, market price transfers are positive and the share of budgetary transfers to producers is already significant (about half of support). Both countries are also in the Eurasian Customs Union.

- Ukraine and Moldova. Both have a negative value of transfers to agricultural producers, especially Moldova with about 20% of the value of production and very limited budgetary support.

- Belarus forms its own group. Substantial and regionally highest budgetary support to agriculture is entirely offset by large negative market transfers, resulting in zero total transfers to agriculture.

The impact of trade policy is mostly modest, but existent, and especially important in specific market and political circumstances. Special measures are export duties and export bans in specific political and economic circumstances (food price crisis, political turbulence). However, it is difficult to recognize the developmental impact of trade policy on production growth and the modernization of agriculture. A special impact on policy and subsequently on trade and partly production is exerted by the visible regionalisation of trade relations, generating new trade patterns, which has started to intensify after 2014. The key processes here are the Eurasian Customs Union and preferential trade agreements with the EU. This alignment of countries is strongly connected to the influence that the Customs Union has on regional trade and to the fact that most of the countries rely heavily on Russia for their exports; the first group therefore promotes exports to Russia and the CU, while for the second this represents a policy issue to be dealt with in terms of diversifying export markets.

This work may be regarded as preliminary, an attempt to increase the body of knowledge about agricultural policy in the CIS 8. Gaps in the literature, weak permanent monitoring systems, complexity of issues and lacking/opaque information and statistics have limited the quality of our work and outputs. We are fully aware of this and wish to stress that the qualitative and quantitative results should be taken with much reserve. This applies in particular to price comparisons, as well as the causal analysis of the causes and effects on prices.

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QUANTITATIVE AND QUALITATIVE ANALYSIS OF AGRICULTURAL POLICY OF ENTITY REPUBLIC SRPSKA (BOSNIA AND HERZEGOVINA)

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Abstract

The aim of this paper is quantitative and qualitative analysis of agricultural policy of entity Republic of Srpska through the use of APM methodology. APM methodology is uniform systematization of agricultural policy measures, which has characteristics of internationally recognized classification (EU and OECD classification). Analysis of agricultural policy of entity Republic of Srpska was related to the period 2000-2013. The agricultural policy had characteristic of production and income-oriented policy, which confirms a large share of funds for direct producer support measures with average of 56% in relation to total budgetary support. In the structure of aggregate budgetary support to agriculture and rural development (TSEb) most important element was budgetary support to producers (PSEb). The analysis of the PSEb structure according to criteria of implementation indicated the dominance of payments based on output, with an average relative share of 56%, while according to second criteria based on the degree of commodity specificity is a producer single commodity transfers with an average share of 54%. Considering that one of the most important tasks in the EU accession process is improving the competitiveness of agriculture, it is necessary the reorientation of existing agricultural policy in the direction of increased support for investments in modernization of agro industry, which would to creating a more efficient sector.

Key words: agricultural policy, APM methodology, entity of Republic Srpska.

Introduction

Considering the complexity of state structure of Bosnia and Herzegovina, which determines the existence of two entities: Federation of Bosnia and Herzegovina and Republic Srpska, as well se District Brcko, and the fact that at the state level there is no common Ministry of Agriculture and Rural Development suggests that the management of agricultural and rural development is under authority of existing administrative units. In other words, entities and District Brcko create their own agricultural policies, which represent medium or long-term programs of agricultural and rural development. Agricultural sector in Republic Srpska is considered as most reputable resource within this entity. In the economy of Republic Srpska agriculture plays an important role in view of its participation in the formation of GDP (11% share of agriculture of total GDP) and employment (30% employment in agriculture in relation to total employment). The importance of this sector in the overall economic development of Republic Srpska was

confirmed through the creation of strategic document – Strategy of agricultural development until 2015, with clearly defined long-term objectives of agricultural development. The strategic document was for the first time adopted in 2006 and on the one side goals should ensure increasing of volume and protection of domestic agricultural production, as well as improving of competitiveness in relation to neighboring countries and EU, on the other hand adjusting of sector to market economy principles and capacity building for inclusion in international integration. By creating of this primary document were set basic principles for defining the objectives, measures and instruments of agricultural policy of Republic Srpska contained in Law on agriculture (Official Gazette, No. 70/2006). Before the adoption of Strategy in 2006, started in 2000 the program of support to agriculture and rural development based on allocation of funds for development of milk, seeds and tobacco sector, as well as rural infrastructure. The second important strategic document at the level of Republic Srpska was Strategic plan for Rural Development (2010-2015), which objectives should achieve increasing of productivity and competitiveness of agricultural sector, balanced rural development, stopping of migration process in rural areas, improving of quality life and revitalization of hilly and mountains areas. In both strategic documents were give detailed action plans for the achievement of defined objectives. Besides these two documents, other laws and regulations consists the legal framework for the implementation of agricultural policy in Republic Srpska. In general, the support to agricultural and rural development was provided by agricultural budget of Republic Srpska. The form, type and scope of budgetary transfers to agriculture, as well as their development over time are important for agricultural policy analysis (Volk *et al.*, 2012). Aiming to quantitative and qualitative analysis of agricultural policy measures of Republic Srpska from the standpoint of analyzing of agricultural budget, in this paper was used APM methodology. Agricultural policy measures at the level of Republic Srpska were primarily classified in accordance to EU classification and then with OECD classification. APM methodology is useful tool because it allows international comparison of agricultural policy, especially in the countries preparing to EU accession with CAP.

Materials and methods

Quantitative and qualitative analysis of agricultural policy measures of entity Republic Srpska has been done on the basis of APM methodology, described by Rednak and Volk (2010). In accordance to same authors, APM methodology is based on a unique classification of agricultural policy harmonized with internationally recognized classifications; EU and OECD classification. According to the EU concept of systematization of agricultural policy measures, the framework for the classification consists of three pillars for support of agriculture and rural development: (1) Market and direct producer support measures, (2) Structural and rural development measures, (3) General measures related to agriculture. In addition to these three pillars of support to agriculture, in the context of the above mentioned classification are also included “Miscellaneous transfers to agriculture”. These are budgetary expenditure on which there is not enough relevant information to allocate them to one of the appropriate category. The first pillar includes measures which contribute to increasing of income of agricultural producers, divided into two groups: (1) Markets support measures and (2) Direct producer support measures. Market support measures incorporate the measures by which the policy

influences the supply and demand on the domestic market, and thereby indirectly influences the prices of agricultural products (Volk *et al.*, 2012). Budgetary expenditures related to this group of measures are disaggregated into three groups: (1) export subsidies, (2) market interventions and (3) consumer support. At the level of Republic Srpska, in analyzed period support to market was exclusively operationalized through budgetary expenditure for market interventions (intervention buying) and consumer support (support to food industry for purchasing of domestic products). Direct producer support measures include two main group of measures: (1) direct payments and variable inputs subsidies and (2) disaster payments and other compensations to producers. Generally, for level of Republic Srpska, budgetary expenditures for measures of direct producer support were dominated in total budgetary support to agriculture. **The second pillar** comprises measures related to structural adjustments and rural development, grouped in three axes: (1) Improving the competitiveness of the agricultural sector, (2) Improving the environment and countryside, (3) Supporting rural economy and population. The first axis is divided into two main groups of measures: (1) on-farm restructuring support and (2) agri-food restructuring support. The most important group of second APM pillar at the level of Republic Srpska in analyzed period was measures related to improving of agricultural sector, precisely on-farm restructuring support. The second axis involves measures related to environmental and landscape payments to producers and environmental payments not directly linked to agriculture. Within this axis at the level of Republic Srpska, the most important payments were other payment with environmental objectives. The third axis is broken down into three groups of measures: (1) Support to rural population directly linked to farms, (2) General support to rural economy and population (3) Building local capacity (LEADER). In period 2000 – 2013, measures of general support to rural economy and population (rural infrastructure and business creation) dominated within the third axis. **The third pillar** includes all measures with the aim to supporting of public services related to agriculture such as research and development, advisory and expert services, food safety and quality control, technical assistant and other general support measures. In period 2000-2013, the most important group of measures within third pillar was food safety and quality control measures. **OECD concept** in classification of total transfers associated with agricultural policy involves the grouping of measures in categories of transfers to producers individually (PSE), transfers to consumers individually (CSE) and transfers to general services to agriculture collectively (GSSE). In accordance to OECD (2010), **PSE** is the annual monetary value of gross transfers from consumers and taxpayers to agricultural producers, measured at the farm gate level, arising from policies that support agriculture, regardless of their nature, objectives or impacts on farm production or income. PSE includes two types of transfers: MPS (market price support) and BOT (aggregate budgetary and other transfers to producers). MPS is defined as the annual monetary value of gross transfers from consumers and taxpayers to agricultural producers, arising from policy measures that create a gap between domestic prices and border prices of agricultural commodity, measured at the farm gate level (OECD, 2010). The procedure of calculating the MPS is very complex and requires the existence of a comprehensive database, which in most transition countries is not available. The lack of MPS database made impossible the estimation of this indicator in this paper, and also limited an overall analysis of agricultural policy of entity Republic Srpska. Therefore the analysis of agricultural policy of entity of Republic Srpska in accordance to OECD classification was exclusively based on

calculating of budgetary components PSE/TSE indicators, **precisely PSEb/TSEb**. The calculation of PSEb/TSEb provided a **partial picture** of agricultural policy of Republic Srpska, but whatever it allows to review the structure of measures. Producer estimated budgetary support for level of Republic Srpska was analyzed on the base of two criteria: (1) **the implementation criteria** and (2) **based on the degree of commodity specificity**. Budgetary transfers to producers in PSE according to implementation criteria are classified into seven categories, depending on the **basis of support** (output – category A.2.; input – category B; area/animal numbers/ receipts/ income – categories C, D and E; non-commodity criteria – category F), whether the support is **based on a current** (categories A.2., B, C, F) **or non –current basis** (categories D and E), or the production is **required** (categories C and D) **or not** (category E). In accordance to second criteria, PSEb is broken down into four transfer groups of support, i.e. support provided to a single commodity (SCT), a group commodity (GCT), all commodities (ACT), or producers are not required to produce some commodity to receive support (OTP). **Consumer Support Estimate (CSE)** is the annual monetary value of gross transfers from (to) consumers of agricultural commodities, measured at the farm gate level, arising from policy measures that support agriculture, regardless of their nature, objectives or impacts on consumption of farm products (OECD, 2010). CSE indicator includes the following transfers: 1) Price transfers from (to) consumers, as the inverted value of MPS, (2) Budgetary transfers to first consumers of agricultural products and (3) Consumer subsidies associated with the programs of market price support for domestic producers. For the analysis of agricultural policy of Republic Srpska were calculated budgetary transfers to consumers from taxpayers (TCT) in 2013. **General Services Support Estimate (GSSE)** presents the annual monetary value of gross transfers to general services provided to agricultural producers collectively, arising from policy measures that support agriculture regardless of their nature, objectives and impacts on farm production, income or consumption (OECD, 2010). According to the nature of the services provided to agriculture, budgetary transfers included in GSSE are divided into seven categories (H. Research and development; I. Agricultural schools; J. Inspection services; K. Infrastructure; L. Marketing and promotion; M. Public stockholding; N. Miscellaneous). **Total Support Estimate (TSE)** is the annual monetary value of all gross transfers from taxpayers and consumers arising from policies that support agriculture, net of the associated budgetary receipts, regardless of their objectives and impacts on farm production and income, or consumption of farm products (OECD, 2010). For the calculating of TSE indicator exist two methods, i.e. by receipts and source. Total estimated budgetary support to agriculture - TSEb in Republic Srpska for period 2000-2013 was calculated in accordance by recipient. Generally, the first method sums up the transfers distinguished by recipient, i.e. transfers to producers (PSE), transfers to general services (GSSE) and transfers to consumers from taxpayers, i.e. consumer subsidies (TCT) (OECD, 2010). Considering that TSEb Republic Srpska is calculated using following formula:

$$TSEb = PSEb + GSSE + TCT$$

In the paper was calculated relative indicator, derived from absolute values of GSSE: i.e. percentage GSSE (%GSSE) as a share of TSE. For the classification of agricultural policy and analysis of total budgetary support to agriculture and rural development of entity Republic Srpska in accordance with APM methodology were used

official data on agricultural budgetary transfers⁴ for period 2000 – 2013 by the Ministry of Agriculture, Forestry and Water Management. In the paper are presented the original research results for period 2000-2011 from doctoral thesis titled: "Possibilities for development of agricultural sector of Republic Srpska in accordance with principles of EU Common Agricultural Policy", by Vesna Mrdalj, PhD, defended in 2014 at the Faculty of Agriculture, University of Banja Luka .

Results and discussion

The total budgetary support to agriculture and rural development in Republic Srpska was in average about 50.70 million KM. Quantitative analysis of budgetary expenditures indicates an increasing of support to agriculture and rural development in period 2000-2008. In 2008 was achieved the highest budgetary support in the amount of 107.80 million KM, which is fourteen times more in compare to the initial 2000. A significant decline of budgetary support to agriculture and rural development from 49% was recorded in 2009 compared to 2008. Renewed growth was recorded in 2010, when totally budgetary support amounted 95.83 million KM. The last three years of analyzed period recorded decline in budgetary support to agriculture (2011. -16%; 2012. -24%; 2013. -37% compared to 2010) (Table.1). In the structure of budgetary support to agriculture and rural development dominated market and direct producer support measures. In average, budgetary expenditures for the first pillar measures amounted 31.68 million KM or 59% related to total budgetary support to agriculture. The maximum allocation of funds for the market and direct producer support measures in the amount of 75.52 million KM were recorded in 2008. Market support was operationalized through the agricultural budget for intervention purchase. Through market institution (Commodity Reserves) was intervened on the grain market , mainly redeemed surplus of wheat, corn and livestock (pigs, cattle and broilers). Budgetary support for the implementation of intervention buying measure was introduced in 2006. The volume of budgetary expenditure for intervention buying was 1.39 million KM, while significant allocation of funds followed in 2010 (8.55 million KM). Budgetary transfers to consumers from taxpayers was recorded in 2013 and referred to support to food industry for purchasing of domestic products. The budgetary expenditures amounted 1.80 million KM.

Within the first pillar of support to agriculture, the most important group represented measures of direct support to producers. This group included payments based on output, current area/animal, variable input subsidies and compensatory payments, which have direct impact on production. In average, budgetary expenditures for direct support to producers amounted 29.78 million KM. Dominated measures within direct producer support were direct payments to producers with an average amount of 22.26 million KM. The most widely used instrument to support agriculture, which has no direct price impact is subsidizing of production per unit of product (Lovre and Zekic, 2011). Direct payments based on output (milk, wheat, tobacco, fruit seedlings) amounted in average of 17.68 million KM. The highest amount of these payments of 32.12 million KM was recorded in 2008. Important place in the multi-year period occupied the direct payments based on current area/animal, with an average amount of 4.57 million KM. Direct payments based

¹ These annual amounts of subsidies represent amount of approved funds for a particular calendar year including the transferred obligations, which are paid from the budget in the coming year.

on current area have an average amount of 2.67 million KM, while the direct support for meat production around 1.90 million KM. Direct support through payments based on current animal number mostly enjoyed producers of beef cattle, pigs and broilers. Variable input subsidies, as a form of support directed to reducing of production costs, in analyzed period occupied an important position within agricultural policy of Republic Srpska. Payments for variable input subsidies amounted in average about 6.95 million KM. The structure of this group consisted of subsidies for plant production (seeds), subsidies for (breeding) animals, fuel subsidies, insurance subsidies and interests concessions for short run loans for agricultural production. Compensatory payments are payments aimed to recovering damages incurred as a result of natural disasters, plant and animal diseases etc. 2010 recorded the largest amount of budgetary funds of 8.00 million KM for compensatory payments, when in the same time in Republic Srpska created the largest volume of damages caused by floods and summer droughts. The Strategic Plan for Rural Development 2010-2015 defined structural and rural development measures. On the basis of socio-economic analysis of situation in rural areas of Republic Srpska, as well as identifying of existing key problems and constraints, the strategic plan defined measures, whose operationalization is needed to ensure achieving of following goals: (1) improving of competitiveness of agriculture and forestry, (2) improving of environment and rational management of natural resources and (3) improving of quality life and introduction of on-farm diversification into non-agricultural activities. In accordance with the set strategic directions, the second pillar measures were classified by axis and thus are fully in line with EU rural development program (Council Regulation (EC) No 1698/2005 on support for rural development by the EAFRD). Budgetary expenditures for second pillar of support to agriculture amounted average 12.34 million KM. The first set of measures within the first axis related to on farm restructuring support were measures for modernization of agricultural holdings with aim to increase productivity and technological efficiency of production. Analysis of support for the restructuring of agricultural holdings is also important from the point of view of its comparison within the measures of direct support to producers. Based on comparison between direct income support and payments for on-farm restructuring support, one can assess whether a certain agricultural policy is more „ income – oriented„ or „ development- oriented„ (Rednak and Volk, 2010). On farm – investment support comprised investments in machinery and equipment, land improvement – irrigation, investments in livestock and plant production, interests concessions for medium and long –term loans for fixed assets on the farm. Budgetary expenditures for on – farm investment support amounted an average of 6.78 million KM. Other on-farm restructuring support has been implemented during the last six years of analyzed period, and was related to co-financing of training and education programs for improving of farmer skills, co-financing of education programs for young farmers as specific target group, introduction of innovative programs in livestock and plant production on farms. An average budgetary support for this group of measures amounted about 0.27 million KM. With the aim to increase efficiency and competitiveness of agri-food sector in Republic Srpska, in period 2003-2013 form agricultural budget were allocated funds for its restructuring. Budgetary support for agri-food restructuring amounted an average about 2.14 million KM. This support referred to investments in modernization of processing sector, construction of collection points for agricultural products and improving of marketing and promotion activities. The implementation of these measures was all the more significant, given that in

some regions of Republic Srpska there were no facilities for the purchasing and processing of agricultural products. In order to overcome the major obstacles in the development of agricultural sector of Republic Srpska, from the standpoint of organization of agricultural markets, as well as the system of purchasing and protection of agricultural producers from competition, was introduced support for organization of agricultural associations and cooperatives. Support to organization of agricultural associations and cooperatives existed since 2005 (an average amount of 0.31 million KM). Second place of second pillar support occupied measures for improvement of quality life and development of rural economy, with an average amount of 3.10 million KM. Budgetary support within third axis was mostly allocated to a group of measures – general support for the rural economy and population, with an average amount of 3.08 million KM. Average budgetary support for measures of second axis – improving the environment and the countryside was 0.04 million KM. The policy of public services related to agriculture in period 2000 – 2013 contained measures to increase production efficiency (research and development), food safety and quality control (veterinary control, plant health control and quality control), marketing and promotion of agricultural products. Budgetary expenditures for the third pillar of support to agriculture and rural development have averaged 6.68 million KM. The most important group was measures for food safety and quality control with an average amount of 3.56 million KM.

SECTION 2. AGRICULTURAL ECONOMICS

Table 1.1. Total budgetary transfers to agriculture and rural development in entity of Republic Srpska (in million KM) 2000-2013.

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Market support measures	0.00	0.00	0.00	0.00	0.00	0.00	1.39	0.00	0.00	0.00	8.55	0.00	2.35	2.65
Direct producer support measures	3.21	8.01	7.54	10.48	17.96	17.96	27.38	42.77	75.02	36.51	41.36	45.84	44.14	39.03
Direct payments to producers	2.98	6.51	5.76	8.12	14.53	13.02	18.96	27.06	69.36	29.23	24.27	27.53	34.10	30.18
Direct payments based on output	2.98	6.51	5.75	7.67	12.79	11.29	15.66	22.38	32.12	26.32	21.24	23.90	31.32	27.68
Direct payments based on current area/animal	0.00	0.00	0.01	0.46	1.74	1.73	3.30	4.69	37.24	2.92	3.02	3.63	2.78	2.50
Variable input subsidies	0.23	1.50	1.78	2.36	3.15	4.95	8.42	15.70	5.66	7.28	9.09	18.31	10.04	8.85
Disaster payments and other compensation to producers	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.00	0.00	0.00	0.00
Miscellaneous market and direct producer support	0.00	0.00	0.00	0.00	0.18	5.24	1.35	0.88	0.50	0.92	0.00	2.55	0.00	0.00
Market and direct producer support measures	3.21	8.01	7.54	10.48	17.86	23.20	30.13	43.65	75.52	37.43	49.91	48.39	46.48	41.68
Improving the competitiveness of the agricultural sector	0.00	0.00	0.01	1.57	2.15	5.59	11.55	9.21	14.66	5.94	21.55	24.66	19.47	12.28
On-farm restructuring support	0.00	0.00	0.01	1.38	1.64	4.75	8.43	7.68	12.06	4.98	17.72	18.28	13.49	8.32
On-farm investment support	0.00	0.00	0.01	1.38	1.64	4.75	8.43	7.68	11.05	4.58	17.00	17.50	13.01	7.91
Other on-farm restructuring support	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.01	0.41	0.72	0.78	0.47	0.42
Agri-food restructuring support	0.00	0.00	0.00	0.19	0.50	0.84	3.12	1.54	2.60	0.96	3.83	6.38	5.98	3.96
Improving the environment and the countryside	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.03	0.16	0.14	0.10	0.10
Environment and landscape targeted payments to producers	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.03	0.04	0.05	0.03	0.04
Agri-environmental and animal welfare payments to farmers	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.03	0.04	0.04	0.03	0.04
Environmental payments not directly linked to agriculture	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.09	0.07	0.06
Other payments with environmental objectives	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.09	0.07	0.06
Supporting rural economy and population	0.00	0.00	0.00	0.22	2.97	1.64	0.32	4.37	4.50	5.94	18.05	1.49	2.50	1.49
Support to rural population directly linked to farms	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.06	0.00	0.05	0.00	0.00	0.00	0.00
Support to on-farm diversification into non-agricultural activities	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.06	0.00	0.05	0.00	0.00	0.00	0.00
General support to rural economy and population	0.00	0.00	0.00	0.22	2.97	1.64	0.27	4.15	4.50	5.89	18.05	1.49	2.50	1.46
Building local capacity (LEADER)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.03
Structural and rural development measures	0.00	0.00	0.01	1.79	5.12	7.24	11.88	13.58	19.20	11.91	39.76	26.28	22.07	13.87
Research, development, advisory and expert services	0.00	0.00	1.17	1.48	1.99	0.72	0.61	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Research and development projects	0.00	0.00	1.17	1.48	1.99	0.72	0.61	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Food safety and quality control	0.30	1.85	1.71	1.93	2.64	2.85	4.19	5.73	11.69	4.25	4.43	3.51	2.51	2.18
Veterinary control	0.30	1.85	1.71	1.81	2.64	2.58	4.04	5.04	11.44	3.99	4.22	3.40	2.32	2.00
Plant health control	0.00	0.00	0.00	0.12	0.00	0.12	0.00	0.16	0.25	0.00	0.21	0.11	0.06	0.10
Quality control	0.00	0.00	0.00	0.00	0.00	0.15	0.15	0.53	0.00	0.25	0.00	0.00	0.13	0.08
Other general support measures	4.06	7.29	6.35	6.47	0.00	0.31	0.36	1.15	1.37	1.93	1.73	2.02	2.22	2.58
General measures related to agriculture	4.36	9.14	9.23	9.87	4.64	3.88	5.16	6.87	13.06	6.17	6.16	5.54	4.73	4.77
Total budgetary support to agriculture	7.56	17.15	16.78	22.14	27.61	34.32	47.17	64.08	107.78	55.51	95.83	80.20	73.29	60.31

Source: Mrdalić, 2014, 2000-2011; 2012-2013: calculation by author.

Total budgetary support to agriculture (TSEb) as the sum of budgetary support to producers, general services related to agriculture and direct budgetary support to consumers in average was amounted 46.53 million KM (Figure 2.). In period 2000 – 2008 TSEb was continually increased, while in 2008 reached a maximum of 103.28 million KM. Drastic decline of TSEb almost for one half (52%) was recorded in 2009 compared to 2008. The most important component of TSEb in analyzed period was PSEb with an average amount of 37.69 million KM or relative share 76%. Support to public services related to agriculture amounted in average 8.73 million KM or relative share 26%. Significant share of PSEb transfers within TSEb indicates the dominance of agricultural policy measures, i.e. budgetary transfers which have an impact on production, consumption, trade and income. Since the PSEb transfers dominated in aggregate TSEb, changes in the values of this indicator induced parallel changes in the value of TSEb.

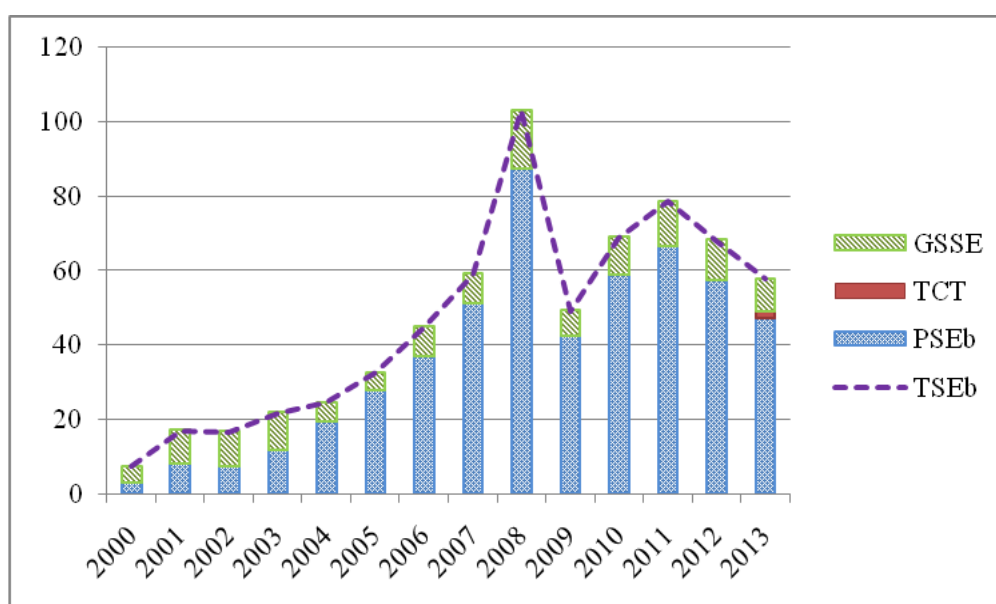


Figure 1. Total budgetary support to agriculture of Republic Srpska (million KM), 2000-2013.
Source: Mrdalj, 2014: 2000-2011; 2012-2013: calculation by author.

Analysis of PSEb based on criteria implementation has shown that the most important category was payments based on output (A.2), with average relative share of 56% (Figure 2.). Payments based on input use (B.) took approximately one third of aggregate budgetary support to producers (average relative share of 33%). Payments based on current A/An/R/I amounted in average 9% of aggregate PSEb. In 2008 this category of payments recorded the highest share of 43% in the structure of PSEb. The dominant share of payments based on output in the structure of PSEb is a result of higher budgetary expenditures to subsidize production per unit of product. Budgetary support for variable inputs subsidies in plant production and on-farm investment support induced a high share of payments based on input use in relation to aggregate PSEb. Direct payments based on current area/ animal were significantly influenced the high share of production-coupled payments based on current A/An/R/I. The structure of PSEb analyzed on the degree of commodity specificity showed that the single commodity transfers had the highest relative

share in relation to aggregate budgetary support to producers (relative average share of 54%). On the second place were all commodity transfers with relative average share of 28% in relation to aggregate PSEb. About 16% of relative average share in in PSEb occupied by the group commodity transfers. In the structure of SCT dominated the transfers for milk, with average relative share of 75%. Transfers for variable input subsidies in plant production dominated in the structure of all commodity transfers, with an average relative share of 37%. The largest part of group commodity transfers in analyzed period referred to transfers for breeding animals (relative average share of 49%) and meat production (relative average share of 20%).

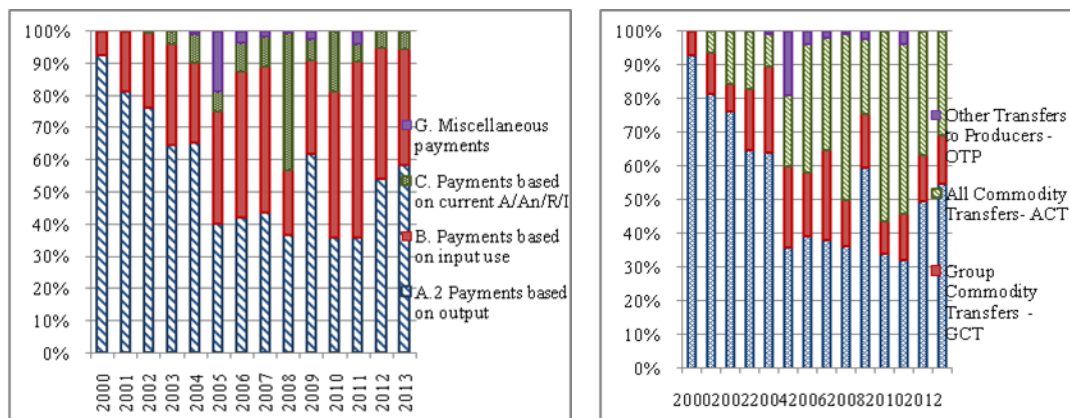


Figure 2. Structure of budgetary producer support (PSEb) for level of Republic Srpska based on criteria implementation and on the degree of commodity specificity, 2000-2013.

Source: Mrdalj, 2014: 2000-2011; 2012-2013: calculation by author.

Conclusions

Quantitative and qualitative analysis of the agricultural policy of Republic Srpska based on the application of APM methodology showed the attributes of policy oriented towards supporting the production and income, as evidenced by a significant share of market and direct producer support measures, precisely measures of direct support to producers. During the analyzed period, direct producer support has been the main element of agricultural budgetary transfers in Republic Srpska. Direct payments based on output had a large share in compare with other form of direct payments to producers. In certain years, direct payments based on current area/animal are significant increasing their share (2008), which is the first step toward the harmonization with the EU CAP and WTO principles. A strategic piece of advice would be that the policy gradually focuses on per head and area payments for those sectors which have been gaining support in the EU since 1992 (Volk *et al.*, 2012). Variable input subsidies occupied a significant share in direct support to producers. Funds aimed to supporting of structural and rural development measures are lower in relation to funds for first pillar measures. In 2003-2007 showed an increasing tendency of funds for structural and rural development measures. These funds were mostly allocated for improving the competitiveness of agricultural sector *i.e.* on –farm restructuring through investment support. Very small shares of funds intended to second

axis- improving the environment and the countryside. Funds aimed to support measures within the group – general support to rural development and population showed significant increasing in certain years of analyzed period. Funds for the implementation of general measures related to agriculture mostly intended for veterinary and plant health control. Applying OECD classification of support to agriculture, in the structure of the aggregate estimated budgetary support for agriculture and rural development (TSEb), budgetary support for producers was the most important element (PSEb). The analysis of the structure PSEb according to the criteria of implementation indicated the dominance of payments based on output (A.2.), while according to the second criteria based on the degree of commodity, a producer single commodity transfers (SCT). The above analysis of agricultural policy of Republic Srpska through APM methodology showed a great difference in compare with EU CAP measures. The measures like direct payments based on output and input subsidies are not a part of support system to producers in EU, while in Republic Srpska they are dominated group of direct producer support measures. In the EU accession prpsca, one of the most important tasks for each country presents improving of competitiveness of agricultural sector. Considering that, in the future decion-makers in Republic Srpska should focus more on increasing of support to second pillar measures on-farm investments, support to investment in food processing industry and organizing of agricultural producers). Also more attention needs to be directed to increasing of support to general measures related to agriculture especially to research and development, extension and advisory services, since these measures long-term have a great impact on strengthening and development of agricultural sector as whole.

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Faculty, University of Ljubljana. Selected Poster prepared for presentation at the International Association of Agricultural Economists (IAAE) Triennial Conference, Foz do Iguaçu, Brazil 18 – 24 August.

UDC: 338.439(497.7)
Preliminary communication

ALIGNED SUPPLY AND DEMAND OF IMPORTANT AGRICULTURAL PRODUCTS IN MACEDONIA

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Abstract

Natural conditions and tradition, influence Macedonian agriculture to be characterized by wide range of products, but often in small periodical series, that have no impact on the market, nor can meet the consumer demand. On the other hand, the demand becomes more versatile and requirements for new production systems associated with food safety obligations, adjusting production to climate change etc. are increasing. To this end, this paper addresses the issues of supply and demand of significant agricultural products in the Macedonian farming sector. The method is based on the view and the comparison of the official statistical indicators as well as own calculations. Namely, there are significant agricultural products what highly exceed the domestic demand, and also equally significant products that can not meet the domestic demand. The analysis will provide better basis for creating of public policy for development of macedonian agriculture.

Key words: market, supply, demand, agricultural products.

Introduction

Alignment between demand and supply of agricultural products is crucial for creating of appropriate agricultural policy for each country. The balance shows what policies should be undertaken for the development of specific productions, how to lead the production towards self-sufficiency of the domestic demand and how to increase competitiveness at the export of excess of certain production. Compiling of the annual balance enables following of production per year, the volume of demand, imports what meet domestic consumption as well as following of the export of surplus products on the domestic market. Balancing of demand and supply for Macedonia is very significant due to insufficient supply of important agricultural products at the domestic market and high dependence on imported food, what contributes to increasing of imbalance between imports and exports in Macedonian economy. Namely, that portion is around 14% and the coverage of the value of imported food to the value of exported is about 54%.

The main objective of this paper is to highlight the necessity for creating of annual balances for some agricultural products, presented for period of three years, for the products that are of big importance for Macedonian agriculture. Furthermore, the aim was to show the possibilities: how the yearly compared annual balance sheets reveals particular

trends of movement as a result of certain policies or production conditions. In line with these tendencies, possible intervention in the future can be done.

Materials and Methods

The research is based on a comparison of official (published) statistical information on: total production for certain products, the quantity of exported products, the quantity of imported products, structure for using of the available production and determining of the satisfaction of total and individual consumption. The example covers the preparation of balance sheets for 8 products as following: wheat, corn, tobacco, tomatoes, apples, grapes, cow milk and beef. The examination covers a period of 3 years (2012, 2013 and 2014). Part of the selected products are important for export since the large volume and high market suficit (tobacco, tomatoes, apples and grapes), and some of the products are with a continuing shortage on the domestic supply and are with the highest volume and value of imported agricultural products, as are: wheat, corn, cow milk and beef. Specific methods what are applied to certain products will be explained in their balance sheets. The important indicator in the balance *self-sufficient* is the result of ratio between production and total demand.

Results and discussion

Balance of supply and demand of wheat

Table 1. Balance of supply and demand of wheat

Indicator	Measurement unit	2012	2013	2014
Production	000 t	215	259	288
Import	000 t	66	66	88
Export	000 t	1	/	3
Available	000 t	280	325	373
Utilisation:				
= for seed	000 t	16	17	15
= for fodder	000 t	11	13	18
= human consumption	kg/capita	150,6	153,8	151,8
Self-sufficient	%	70,0	82,2	92,0

In the last 3 years, the total wheat production is increased by 20,5% and 33,9%, according to base year 2012. The total consumption is about 300 thousand tons, what shows that with a relatively small increase in yields, the total needs can be meet, because already in 2014, self-sufficient indicator reached 92% of the total needs for wheat (Table 1). Consumption per capita is multiplied by the number of inhabitants and with the ratio of total production or total demand is calculated the self-sufficing proportion. Products from wheat are calculated with coefficients and converted into the primary product.

Balance of supply and demand of maize

Table 2. Balance of supply and demand of maize

Indicator	Measurement unit	2012	2013	2014
Production	000 t	116	131	137
Import	t	442	418	183
Export	t	15	12	51
Available	000 t	116	131	137
Utilisation:				
= for seed	t	1,168	1,241	1,220
= losses	t	580	582	583
= for fodder	kg/capita	115	130	135
Self-sufficient	%	98,8	98,9	98,8

In the balance of wheat and maize, there are similarities in terms of structure, because in the balance is included consumption (projected) of seeds. Whether the seed is imported or is from own production, it takes appropriate place in the balance, and is calculated on the basis of the norm for the respective crop per unit capacity. Data for maize (Table 2) displays an increase in total reproduction, with differences in 2013 and 2014 compared to 2012, from 13.0% and 18,1% respectively. Export volumes are relatively small, but in 2014 the exported amount is becoming close to 30% of the imported amount. The balance encourages which show a high degree of self-sufficient and relatively small shortage of maize in the domestic market.

Balance of supply and demand of tobacco

Table 3. Balance of supply and demand of tobacco

Indicator	Measurement unit	2012	2013	2014
Production	t	27,333	27,859	27,588
Import	t	6,516	5,900	6,220
Export	t	25,690	25,920	26,197
Available	t	8,759	7,839	7,631
Personal consumption	kg/capita	5,4	4,5	4,8
Self-sufficient	%	312,1	353,4	361,3

It is known that tobacco in Macedonia is produced mainly for export, because the exported quantity accounts over 90% of production. The tobacco industry also indicates increased production trend as a result of growing interest among farmers for bigger production due to the stimulative financial support from the state. The balance shows that Macedonia produce over 3,5 times larger quantities of tobacco than the needs of the domestic tobacco industry. According to individual consumption of tobacco per capita (Table 3), the official statistics data demonstrates reduction in personal consumption from 5,4 kg/capita to 4,5 kg/capita (2013) to 4,8 kg/capita (2014).

Balance of supply and demand of tomato

Table 4. Balance of supply and demand of tomato

Indicator	Measurement unit	2012	2013	2014
Production	000 t	146	131	161
Import	000 t	2,1	2,1	3,4
Export	000 t	32	28	29
Available	000 t	116,1	105,1	135,4
Self-sufficient	%	125,8	124,7	119,3

Tomatoes are one of the most common vegetables in Macedonia. This product is important for the economy because of permanent exercise of market surplus over domestic consumption and export in relatively large quantities. The production in the examined three years is not stable, because in 2013, the total production is lower than in 2012 for amount of 15,000 t (Table 4). The balance also shows a decline in self-sufficiency in the last 2 years for about 5%. The available quantity of tomatoes expressed in kg/capita is 56,6 (2012) 51.3 (2013) and 66,0 (2014).

Balance of supply and demand of apples

Table 5. Balance of supply and demand of apples

Indicator	Measurement unit	2012	2013	2014
Production	000 t	127	112	96
Import	t	285	382	491
Export	000 t	85	77	29
Available	000 t	42,4	35,4	67,5
Self-sufficient	%	299,5	345,6	142,6

Apple is an agricultural product which is in expansion last ten years, due to establishment of new orchards on relatively large surfaces. Nonetheless, other important fruit species are shortage at the domestic market, such as pears. Due to the large market surpluses in recent years, farmers have problems with sales, therefore serious farmers grievances are appeared. The main reason for the problems with market of the products is unorganized farmers, underdeveloped infrastructure (cold storage) and etc. as well insufficient support, or inadequate logistics for export of apple. Today in Macedonia, from all fruit species of bearing trees, apple orchards represent 55%. The balance shows that a relatively small percentage of the total reproduction is exported (Table 5).

Balance of supply and demand of grapes

Table 6. Balance of supply and demand of grapes

Indicator	Measurement unit	2012	2013	2014
Production	000 t	240	292	196
Import	t	181	231	92
Export	000 t	37	36	27
Available	000 t	203.2	256.2	169.9
Self-sufficient	%	118,1	114,0	115,4

The total production of grapes last years is declining, as well the imports and exports, and the self-sufficient indicator is in close relations (114% - 118%). The indicators for overall production are unexpected, given that the financial support of viticulture is relatively high. In the public, the responsible authorities demonstrate data that there is an expansion in the establishment of new vineyards, however, that is not registered by the official statistics. In 2014, the total production declined by about 33% compared with 2013 (Table 6).

Balance of supply and demand of cow milk

Table 7. Balance of supply and demand of cow milk

Indicator	Measurement unit	2012	2013	2014
Production	million l	349	381	387
Import	million l	3,5	13,7	16,0
Export	million l	1,8	2,7	3,7
Available	million l	347,3	392,0	399,3
Personal consumption	l/capita	139	141	140
Total consumption	million l	285	289	287
Self-sufficient	%	122	132	135

The production of cow milk rises for a long period as well in the last 3 years. As a result of increased personal and total consumption, the exports and imports are also increased. It is known that relatively large quantities are exported as dairy products, like sour milk, yogurt, cheese, butter, cream and so on. which by using of appropriate ratios are converted in fresh cow milk. The balance shows that domestic production exceeds domestic or total consumption by 22% - 35% (Table 7). Movement of increased production is a result of the expansion of higher yielding breeds of cattle and their breeding systems which introduced EU standards.

Balance of supply and demand of beef

Table 8. Balance of supply and demand of beef

Indicator	Measurement unit	2012	2013	2014
Production	t	4,982	5,485	5,522
Import	t	7,652	7,049	6,162
Export	t	/	/	/
Available	t	12,634	12,534	11,684
Personal consumption	kg/capita	7,9	7,9	8,4
Total consumption	t	16,195	16,196	17,220
Self-sufficient	%	30,8	33,9	32,1

Beef is a product of a constant shortage in the domestic market. Self-sufficient indicator is the lowest compared to all other scarce agricultural products in Macedonia, and is just over 30%. In recent years, the rising of beef production is negligible and does not reflect positively on the problem of self-sufficient (Table 8). The comparison between production and imports demonstrate that imports are over domestic production and also reaches about 30%.

Conclusions

Statistical analyses and studies of supply (total production), demand (consumption), exports and imports of some important agricultural products in the Republic of Macedonia lead to several conclusions:

- Three-year trends in the balance of eight important agricultural products show different trends. It is required balance sheet to become a regular activity of the society with in-depth information in order to reveal the actual performance, not only for production, exports and imports, but also for consumption, processing and balancing between the primary and final products.
- There are real opportunities for self-sufficiency in the domestic consumption for main cereals what are the main subject of research since the domestic supply is increasing as a result of increased yields despite declining of areas (eg. wheat), on account of new technologies, varieties, etc.
- The appropriate targeting of agricultural policy is required, especially in animal husbandry, in order to overcome the shortage of livestock products, especially beef, with the aim of reduce import dependences.

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THE INFLUENCE OF OPTIMAL CRITERIA ON THE LEVEL AND STRUCTURE OF VARIABLE COSTS IN CATTLE PRODUCTION

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Abstract

The planning of the cattle production is a complex process into which more criteria are considered. The linear programming is an auxiliary tool which is often used in the agro-economy studies of the cattle production. In this paper a model of a dairy farm for milk production is presented. By using the linear programming, the model is designed according to four optimal criteria: maximization of gross-margin (Variant 1), maximization of agricultural land usage (Variant 2), maximization of ground of leguminous (Variant 3), minimization of spent labor force (Variant 4). In the paper, the compared total sums of variable costs are given, calculated through various optimal criteria, as well as the amount of certain types of costs within the total variable costs. The total variable costs are of lowest level in Variant 1, whereas the highest sum of total variable costs is ascertained in Variant 4 which was for 11.1% higher in comparison to Variant 1. All included parameters pointed to variability, which is easily connected with the applied optimal criteria.

Key words: optimization, model, variable cost, linear programming.

Introduction

Cattle production is one of the most important parts of agriculture in Bosnia and Herzegovina. The planning process in this production is very complicated with a lot of dimensions. Manager cannot respect only one criterion in planning processes. For the optimization livestock production a lot of authors use linear programming model (Costa and Rehman, 2005; Veysset et al., 2005; Crosson et al., 2006; Havlik et al. 2006; Oltjen and Ahmadi, 2006). Some authors presented holistic approach as the most important way in optimization models in agriculture (Krstic and Smiljic 2003, Neal et al. 2006).

This method is not yet massive applied in the process of farm management, but is expected to develop new software with the purpose of supporting to farm management, which computational basis will be the linear programming and / or other similar methods (Vico et al. 2014). In this paper we created linear programming model for one year time

horizon plan. Four criteria were used in objective function: maximization of gross-margin (Variant 1), maximization of agricultural land usage (Variant 2), maximization of ground of leguminous (Variant 3), minimization of spent labor force (Variant 4). Seven results were compared: total variable costs, share of seed costs in total variable costs, share of mineral fertilities costs in total variable costs, share of diesel costs in total variable costs, share of other variable costs in total variable costs, share of feed costs in total variable costs and price of daily meal for dairy cows. The use of linear programming in livestock management involves some steps: describe of research object system; creating logistic model; creating mathematical mode; solving mathematical model; checking optimal solution(s).

The objective of this paper is to describe how optimal criteria have influence on level and structure of variable costs in cattle production.

Materials and methods

The linear programming model was created under few main assumptions:

Maximum arable land 45 ha; maximum stall capacity is 60 spaces for dairy cows; farm can produce wheat, maize, barley and alfalfa; milk yield is 5.500 kg/dairy cow/year. Farm has possibility for purchasing all feed on market and producing some feed on farm. Numerous groups of variable were involved in model: livestock production, crop production, animal feed from market, other inputs from market, labor force, and final production from livestock production.

Objective function:

$$(\max) f = \sum_{i=1}^p \sum_{j=1}^q c_{ij} x_{ij}$$

Constraints:

$$\sum_{i=1}^p \sum_{j=1}^q a_{ijkl} x_{ij} \leq \geq u_{kl} \quad k=1,2,\dots,r \quad l=1,2,\dots,s$$

Conditions of non-negativity:

$$x_{ij} \geq 0 \quad i=1,2,\dots,p \quad j=1,2,\dots,q$$

Indexes:

p – number of group activities

q – number of activities in a group

r – number of group limitations

s – number of limitations in a group

Activities:

x_{ij} ; $i=1,2,\dots,p$ $j=1,2,\dots,q$

Constraints:

u_{kl} ; $k=1,2,\dots,r$ $l=1,2,\dots,s$

Coefficients in a objective function:

c_{ij} ; $i=1,2,...,p$ $j=1,2,...,q$

Coefficients in constraints:

a_{ijkl} - the quantity of j^{th} - activities within a i^{th} - group of activities l^{th} - constraints i r^{th} - group of limitations

Groups of activities: lines of farm production $i=1$ $j=1$; fodder production at a farm $i=2$ $j=1,2,...,8$; dry substance from the concentrated part of the meal $i=3$ $j=1,2$; purchased fodder $i=4$ $j=1,...,19$; other purchased inputs $i=5$ $j=1,...,7$; other variable expenses $i=6$ $j=1$; labor $i=7$ $j=12$; final products $i=8$ $j=5$

Groups of constraints: capacities $k=1$ $l=1,2$; crop rotation $k=2$ $l=1$; balance of nutritive $k=3$ $l=1,...,13$; minimal i maximal components quantity in a meal $k=4$ $l=1,...,12$; balance of other inputs $k=5$ $l=1,...,8$; balance of labor $k=6$ $l=1,...,12$; available labor $k=7$ $l=1,...,12$; balance of mechanization $k=8$ $l=1,...,4$; balance of finished products $k=9$ $l=1,...,5$.

Results and discussion

The results of four variants were analyzed in this paper. Linear programming model provides a lot of indicators of cattle production. Managers can analyzing results in two stages: optimal and post optimal analyze. For example, under optimal analyze of cattle production linear programming models, managers can see data about level of gross margin, optimal number of dairy cows, structure of livestock production, structure of crop production, type and amount feed from market, type and amount other inputs for livestock and crop production, structure of meal for animals, labor force, mechanization use.... Some indicators we can read direct from optimal solution and we must take additional calculations for other indicators.

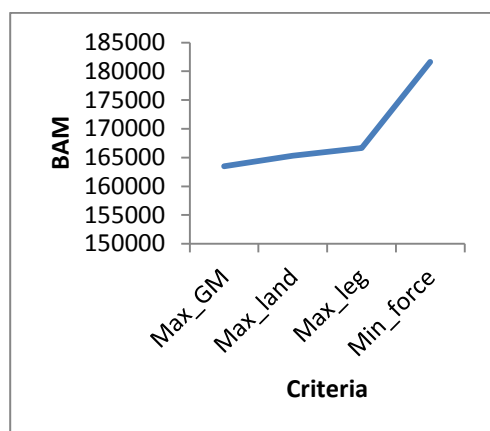


Figure 1. Total variable costs per variants (BAM)

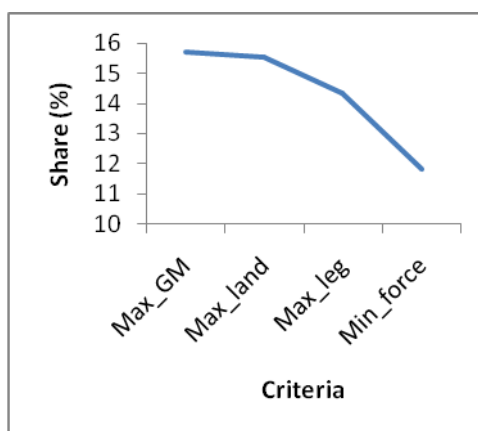


Figure 4. Share of diesel costs in total variable costs

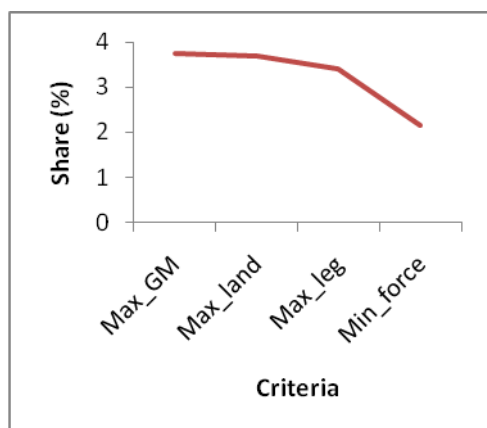


Figure 2. Share of seed costs in total variable costs

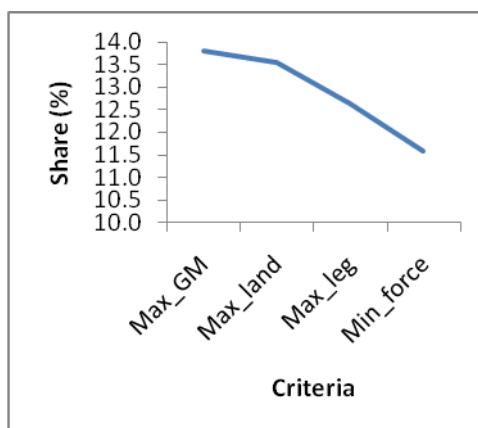


Figure 5. Share of other variable costs in total variable costs

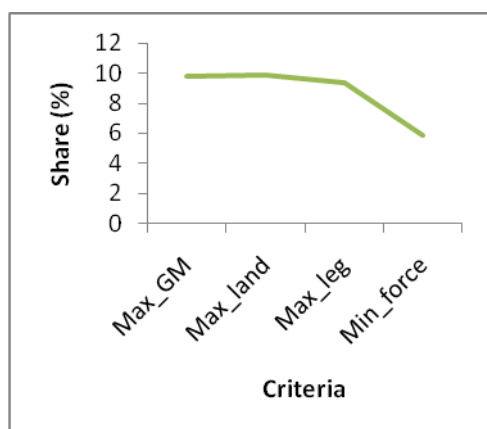


Figure 3. Share of mineral fertilities costs in total variable costs

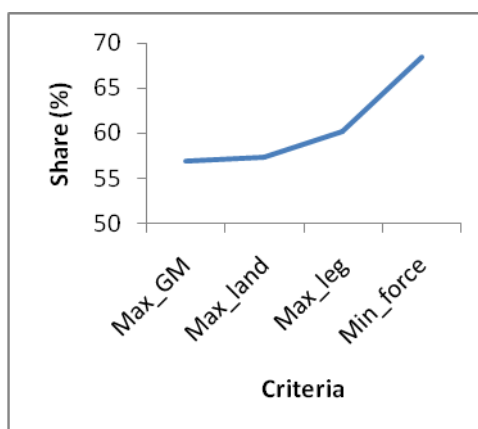


Figure 6. Share of feed costs in total variable costs

Total variable costs have different amounts per variants. The first variant has the lowest variable costs (163.470 BAM⁵), while the fourth variant has the highest variable costs, for 11,1 % more than first variant (Figure 1). This trend follows optimization criteria. The criterion of the first variant is maximization gross margin and the criterion of the fourth variant is minimization labor force. The lowest variable costs in the fourth variant are “price” for its criterion. The seed costs are the highest in variant 1, while they are the lowest in variant 4 (Figure 2). Variant 4 has more than 40% lower seed costs than variant 1. This difference is result where variant 4 uses lower arable land (total 31,03 ha) while variant 1 uses 44,01 ha. Costs of mineral fertilizers are following seed costs, with small difference where variant 2 has higher costs of mineral fertilizer than variant 1. This is very small difference, only 0,61 %, while variant 2 has higher costs for 68,25 % than variant 4 (Figure 3).

⁵BAM – Bosnian mark

Costs of diesel and other variable costs are following above mentioned costs (Figure 4 and Figure 5).

Animal feed costs represent the most significant component of variable costs in dairy production. Manager can improvement economic performance of dairy production through reductions feed costs. Price of meal in particular must be important part of all planning process in dairy production.

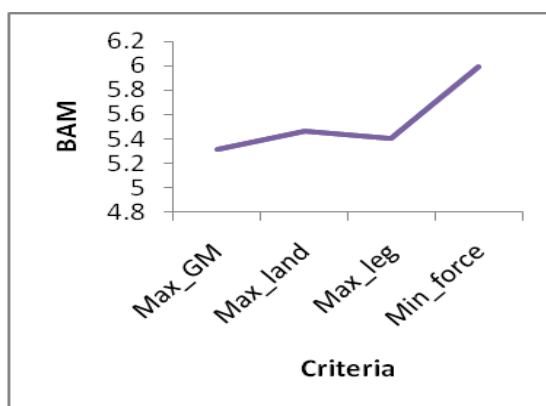


Figure 7. Price of daily meal for dairy cows

Variant 1 has the lowest price of daily meal for dairy cows (5,31 BAM). As expected, variant with minimization labor force in objective function has the highest price of daily meal for dairy cows (Figure 7). These costs are higher for 12,81 % than costs in variant with maximization gross margin as criteria in objective function.

Conclusions

The linear programming model that was created allows use more optimization criteria. The different criteria give different results. First variant with gross margin as criterion has the lowest total variable costs, while variant four with minimization labor force has the highest variable costs. The first variant has the lowest share of feed costs in total variable costs while fourth variant has the highest. Other costs showed variability dependent of optimization criteria. Daily meal for dairy cows has the lowest price in the first variant while fourth variant has the highest, for 12,81 % higher than first variant. The results are expected considering the optimization criteria which we used.

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UDC: 332.72:631.11(497.7)
Original scientific paper

CAPTURING THE FARMLAND MARKET DATA IN THE REPUBLIC OF MACEDONIA

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Abstract

The capital farmland market in the Republic of Macedonia is underperforming and lacks of transparency. The limited availability for pricing information on farmland hinders the establishment of a functioning and transparent land market. Even though farmland markets typically exhibit a low rate of transactions, the land price may vary due to different factors. Hence, the prime aim is to detect initial records on the farmland prices, and to explain the influence of the factors that may affect the farmland price. The data collection is based on the desk and field research methods. The desk research was based on data reported in the Official Gazettes of the RM during the period from January to December 2013, resulting in 1100 observations on the price of arable land and gardens. The field survey covered 244 respondents and was conducted by using the focus group approach during February 2014. The questionnaire included two points for discussion: 1) The lowest, mid and the highest price of the landmark i.e. the hypothetical property, and 2) The factors that influence the price. The results show that there are differences in the observed farmland prices resulting from the desk and field research. The average farmland price resulting from the desk research is 1 €/m², while the one from the field survey is 1.53 €/m². However, there are differences in the prices between regions as well, due to different factors affecting the farmland price. Density showed the strongest positive correlation with the farmland price, meaning that higher density increases the price, and vice versa. Other factors influence the farmland price as well. These initial results shall serve as a basis for future development of permanent records on the farmland price data, which are necessary to persuade timely information on the farmland market value and ease the farmland transactions.

Key words: farmland prices, initial records, factors' analysis.

Introduction

Background information

Farmland is an important asset for every economy. It is used for producing food and other materials necessary for the peoples' well being. Like every other assets, farmland has its own value. Farmland is capable of producing a continuous stream of agricultural services and if it is properly managed, it does not depreciate; however it changes over time

(Gardner and Nuckton, 1979). These changes in farmland occur through drainage, irrigation and conservation structures. Buildings, fences and other improvements also add to the farmland value. However, farmland valuation is not an easy task since there are many other factors related with the value of the farmland. Moreover, it has a market value created throughout market transaction.

The farmland market consists of the sellers (the supply) and the buyers (the demand) of a farm real estate, which are the key drivers of the farmland market value. The sellers are the owners of farmland that are selling their property due to diverse factors mainly driven by farm abandonment or lack of interest to engage in agriculture on a inherited farmland, etc. The buyers of farmland are the existing farmers that expand production, investors and new farmers interested in farm business activities, non-farmers that are attracted to rural areas, etc. Reynolds (1966) emphasizes that all participants on the land market (landowners, prospective buyers, tax assessors, etc.) must place a value on farmland.

The level of prices at which ownership rights in farmland are exchanged is a major determinant in the use of the farmland and in the distribution of economic benefits resulting from its use. Therefore it becomes important to understand the factors that determine farmland prices. Thus, it should be considered that price and value are usually measured to be equal under conditions of perfect competition. However, under actual conditions of the farmland market, prices may be quite different from value.

Definition of the Problem

The capital farmland market in the Republic of Macedonia (RM) is underperforming and lacks of transparency. The limited availability for pricing information on farmland hinders the establishment of a functioning and transparent farmland market.

Even though farmland markets typically exhibit low rate of market transactions, the farmland price may vary due to different factors. Therefore, it is necessary to determine the key drivers of the farmland market in the country since there is no similar official analysis. The necessity for a functioning and transparent farmland market in the RM urged the need for capturing the farmland market data in the country. This necessity is becoming increasingly vital within the Macedonia's reforms in its aspiration to join the European Union (EU).

Aim and Outline

Permanent records on the farmland price data, as well as identification of factors affecting the farmland price, are necessary to persuade timely information on the farmland capital market value. As the farmland is being an important capital asset for every economy, these records and information are needed to ease the farmland transactions in the RM and to provide basis for recurrent farmland market analyses. Thus, the *prime aim* of this article is to: *a)* detect initial records on the farmland prices upon which future valuation of farmland shall be based, and *b)* to identify the possible influence of certain external factors on the farmland price.

The following section presents literature review related with the characteristics of the farmland market and determinants on the farmland value which shall serve in future attempts for farmland valuation in the country. The next section describes the research method in details. The results are then presented followed by a short discussion and conclusions.

Literature review

Economic Value of the Farmland

Farmland is an immobile recourse and has an infinite life, which with proper maintenance may change over time but very slowly (Barry *et al.*, 2000). In contrast to other production factors in agriculture, farmland has special treatment in institutional arrangements such as taxation, government programs, etc. (Barry *et al.*, 1983). Different factors affect farmland values, such as the availability of water, minerals, oil, buildings, as well as conservation practices, etc. Also, farmland values are influenced by particular factors typical for the potential buyers, such as the ownership of agricultural machinery to the potential buyers and preparedness to buy new one (Plantinga and Miller, 2001). And lastly, there are monetary factors affecting farmland values, such as family tradition, hobby farming, rural living and other (Borchers, Ifft, and Kuethe, 2014).

Farmland acquires its economic value from a series of anticipated net returns which will become available over a period of years (Reynolds, 1966). In this regard, farmland has economic value for the same reasons as other goods. Since farmland has greater durability as a production factor than many other goods, the future earning capacity of land becomes important for the purpose of valuation. Theoretically, the market value of farmland should always equal the present worth of its future net returns discounted back to the present (Barry and Ellinger, 2012).

Characteristics of the Farmland Market

The farmland market has certain distinctive features because of the peculiar characteristics of land and the specificities of the agricultural production. In this regard, the farmland market does not have the usual characteristics of a purely competitive market (Colyer, 2004). The characteristics of a purely competitive market are (Kohls and Uhl, 2002): 1) existence of a homogeneous product, that is, the product of each seller is identical with that of every other seller; 2) there are many buyers and sellers in the market and the sales and purchases of each individual are small in relation to the aggregate volume of transactions; and 3) there is free entry into and exit from the market for both buyers and sellers. A perfectly competitive market differs from a purely competitive market. In addition to the above characteristics, both buyers and sellers possess perfect knowledge in a perfectly competitive market.

Even though agriculture is often referred to as purely competitive industry, a different situation occurs in the market for farmland (Reynolds, 1966). Land is a heterogeneous resource that varies greatly in quality. Land is very difficult to classify because of the subjective nature of the classes. For example, the Agency for Real Estate Cadastre in the Republic of Macedonia classifies farmland as I, II, III and IV class. However, two class I farmlands could be quite different under the Cadastre. In addition, the Cadastre's classification is not yet adapted to a comparable classification of the farmland, however improvements are in progress. In the farmland market there is not always a large number of buyers and sellers present. These conditions may affect the characteristic of free entry into and exit from the market. The availability of credit is a factor that may also affect free entry into and exit from the market. If credit is not available, a person without a large cash reserve may be unable to buy farmland. The farmland market does not meet the requirements for a purely competitive market. Imperfect competition results since farmland

is a heterogeneous recourse and there may be a limited number of buyers and sellers in the market. The average buyer and seller only participate in the farmland market occasionally, and as a result their experience is limited. The seller's decision to sell is often connected with some outside event such as the desire to obtain a larger farm, abandonment of the farm or migration to another city or country. On the other hand, from the buyer perspective, there are large considerations involved in most farmland transactions, including the preparedness to undertake great investment farming activities.

The fixed location of the resource is another characteristic of the farmland market. Farmland must always be sold where it is located. The fixed location of farmland tends to localize the market for it.

Materials and methods

In regard to the aim of this study, the research method comprises field and desk research techniques so to provide the initial data on the farmland prices in the Republic of Macedonia. Also, secondary sources were consulted so to examine the factors which affect farmland values in the country, whose relationship with the farmland price was additionally tested.

Field research

The field survey covered 244 respondents and was conducted in all eight regions in the RM during February 2014, by using the focus group approach (a group interview). In this case, the focus groups consisted of 6 villagers (respondents) on average. This approach provides in-depth and in-time information, and by this way it is a cost-efficient for the surveyor. Moreover, group interviews provide the possibility to encourage discussion among participants (respondents) in the focus groups on matters which would be otherwise missed in an individual questionnaire. In most instances, the surveyors utilized the possibility to meet in their home environment in order to induce comfort and confidence of the respondents.

The questionnaire included the two points for discussion: 1) The lowest, mid and the highest price (in Euros/m²) of the landmark i.e. the hypothetical property, that does not have the potential to be converted into construction land in future (a farmland that is about 1 km away from the village and is partially accessible by paved road), and 2) Factors that influence the price such as infrastructure (irrigation, paved road, demography, etc.).

At the beginning of each session (meeting), the surveyor gave introduction for the purpose of the survey and the expected discussion. In this regard, the following questions were used to initiate the discussion about price of each landmarks: 1) If the *landmark* property described below existed in the locality, how much would the farmer (or other respondent) expect to pay for it in the open market?, and 2) If the *landmark* property was owned by the farmer (or other respondent), how much would they be prepared to sell it for?.

Before the field survey was initiated, there was a test survey conducted in a village in the Skopje region to a group of nine farmers. The findings from the visit were the base for the additional instructions for the surveyors.

Desk research

The desk research is based on data that were reported in the Official Gazzetes of the RM (OG) during the period from January to December 2013, resulting in 1100 observations on the price of arable land and gardens for all eight regions throughout the country. Observations that were reported as the price of arable land, but were in fact prices for construction land, were not considered in the analysis.

The data set created from the desk research data covers the following information on the price of the agricultural land: 1) General information on the source (number and date of the issue of the OGs); 2) Legal information on the agricultural land announced for sale (municipality, spot, cadastral municipality, number of cadastral parcel, class of cadastral crop, area on sale (in m²), and 3) Sales price of the arable land and gardens (in Euros/m²).

However, the results from the desk research presented in next section include the average price of the arable land and gardens by region and by municipality, and additionally give an overview of the number of observation by region and municipality. The number of observations per region is different. More than a half of the observations belong to Pelagonia and Southeastern region since there are a large number of observations for Prilep and Strumica, respectively. Also, not all municipalities within a region were covered since there was not any land on sale during the observed period as registered in the OGs.

Data analysis

Since the prime aim of this study is to obtain initial records on the farmland prices in the RM, the analysis is based on calculating average prices on regional level and on a country level. For these purpose mean statistics was employed to data observed from both desk and field research. Additionally, the differences in the observed average prices on agricultural land, which resulted from both data sources, were calculated.

In order to determine whether particular factors affect the farmland price in the country, a correlation statistics was employed. Since all the variables are of interval-scale type, they were analyzed with the Pearson's correlation coefficient. The following factors specific for each region in the country were tested if they influence on the local farmland price: a) Density, b) Arable land per capita, c) Population over 65 years, d) Local road network development, e) Water supply, f) Installed electricity, and g) GDP per capita. The official statistical data on each factor was obtained from the State Statistical Office of the Republic of Macedonia.

Results and discussion*Field research results*

The testing of the questionnaire was conducted in a village of Pobožje in the Skopje region and the focus group covered nine farmers. During the discussion, it was noted that farmers faced difficulties to visualize hypothetical landmark properties. However, initial results from the trial of the focus group approach were obtained. That are, the size of the land that was traded in the surrounding of this village were between 0.2 to 0.5 hectares because part of the village area is very steep. Furthermore, households own 2 ha land on average which is divided in several pieces in different locations. This village did not have any irrigation systems and the average reported price by every discussant was 0.5 €/m². Farmers stated that this price was mostly influenced by the proximity of the parcel to the main road or to

the house of the buyer. The results from the test showed that there should not be further difficulties for farmers to define the hypothetical landmark property, therefore there were no altered instructions given to the rest of the surveyors prior their interviews.

Data on the farmland prices observed from the field survey for all eight statistical regions (the Vardar region, the Eastern region, the Southwestern region, the Southeastern region, the Pelagonia region, the Polog region, the Northeastern region and the Skopje region) are presented in Table 1. The field research data on the farmland prices are based on the opinions on 244 farmers from 42 villages in 37 municipalities. These data are based on the highest, average and the lowest observed price in one village. The group interviews gave different prices for the farmland throughout the country. But, there were no big differences in the range in prices stated by the people from one focus group in each village. Usually, these prices of farmland varied due to several factors. In the regions without irrigation system, typically the mountainous regions in the western part of the country (mainly Polog region), the size of the parcel and the proximity to the road and/or to the village influenced the price of the farmland. Conversely, in dry areas, the proximity of a river or irrigation systems play much bigger role in the determination of the farmland prices. Intensive irrigation is especially essential for the regions characterized with intensive vegetable production. In general, the frequency of the transaction on the farmland market could be also considered as a factor that affect the level of farmland prices, however there are certain difficulties for these price changes to be observed due to the existing imperfection of the farmland market in the country. From a demand perspective, farmland is usually transacted if farmers are willing to enlarge their production (generally from the neighbor), and, from a supply perspective, farmers are willing to sell land so to cover their liabilities to external party.

Table 1. Summary results on the farmland prices based on a field survey data

Region	Number of municipalities	Number of villages	Number of participants	Farmland price (€/m ²)		
				Low	Mean	High
Vardar	5	5	29	0.36	0.72	1.24
Eastern	5	5	29	0.40	1.20	1.90
Southwestern	5	5	28	1.12	2.55	4.00
Southeastern	4	5	28	0.48	0.72	1.10
Pelagonia	4	4	24	0.45	0.70	1.35
Polog	4	7	34	1.05	2.40	3.84
Northeastern	5	5	37	0.72	1.14	1.70
Skopje	5	6	35	1.38	2.83	4.16
Total/ Average	37	42	244	0.75	1.53	2.41

The lowest farmland price is observed in the *Vardar region* due to the low price observed in Caska village that ranges from 0.2 to 0.5 €/m². This village is lowland and the main agricultural production is field crop. The highest price in this region is observed for the village Dolni Disan, which ranges from 0.5 to 0.6 €/m². However, this price would be higher if there is an irrigation system and if it is near a paved road. Also, if the observations include vineyards or orchards, the average price would reach 1.2 €/m². The highest price of 4.16 €/m² is observed in the *Skopje region* since the villages are located

near the capital of the country and are characterized with a well developed local road network, irrigation system, available electricity, etc.

Desk research results

The results from the desk research cover 1100 observations on the average farmland prices (the price for arable land and gardens) and the number of observations by region and municipality. In general, the average farmland price in the RM is around 1 €/m². The highest average price of 3.42 €/m² is observed in the Skopje region, while the lowest of 0.39 €/m² in the Eastern region.

For more details, Table 2 presents the data on the farmland prices observed with the desk research. The average price in the *Vardar region* is 0.71 €/m² and is based on total of 89 observations for four municipalities. The highest average price of 1.29 €/m² within this region is observed for Veles, and the lowest of 0.35 €/m² for Demir Kapija. The average price in the *Eastern region* is 0.39 €/m² and it is the lowest observed regional price. It is based on total of 40 observations for five municipalities. The highest average price of 0.61 €/m² within this region is observed for Kocani, and the lowest of 0.11 €/m² for Stip. The average price in the *Southwestern region* is 1.60 €/m² and is based on total of 90 observations for four municipalities. The highest average price of 1.83 €/m² within this region is observed for Struga, and the lowest of 1.13 €/m² for Debar. The average price in the *Southeastern region* is 0.51 €/m² and is based on total of 250 observations for three municipalities. The most of the observations within this region are for Strumica or 92%, where the highest average price of 0.77 €/m² is observed. The lowest average price of 0.29 €/m² is observed for Valandovo, however based on three observations. The average price in the *Pelagonia region* is 0.47 €/m² and is based on total of 394 observations for four municipalities. The most of the observations within this region are for Prilep or 73% where the price of arable land and gardens is on average of 0.24 €/m². The highest average price of 0.86 €/m² within this region is observed for Resen, and the lowest of 0.10 €/m² for Krusevo which is the lowest observed price within the whole country. The average price in the *Polog region* is 2.19 €/m² and is based on total of 101 observations for two municipalities. The average price is higher in Gostivar (2.66 €/m²) then in Tetovo (1.73 €/m²). The average price in the *Northeastern region* is based on only one municipality of Kumanovo. The average price is 1.43 €/m² and is based on total of 62 observations.

Table 2. Results from the desk research on the farmland prices

Region	Municipality	Total number of observations	Average price (€/m ²)
<i>Vardar</i>		89	0.71
	Veles	23	1.29
	Demir Kapija	2	0.35
	Kavadarci	35	0.68
	Negotino	29	0.55
<i>Eastern</i>		40	0.39
	Berovo	2	0.56
	Vinica	27	0.37
	Delcevo	8	0.32
	Kocani	1	0.61
	Stip	2	0.11
<i>Southwestern</i>		90	1.60
	Debar	2	1.13
	Kicevo	22	1.78
	Ohrid	40	1.67
	Struga	26	1.83
<i>Southeastern</i>		250	0.51
	Valandovo	3	0.29
	Gevgelija	17	0.46
	Strumica	230	0.77
<i>Pelagonia</i>		394	0.47
	Bitola	69	0.68
	Krusevo	7	0.10
	Prilep	288	0.24
	Resen	30	0.86
<i>Polog</i>		101	2.19
	Gostivar	37	2.66
	Tetovo	64	1.73
<i>Northeastern</i>		62	1.43
	Kumanovo	62	1.43
<i>Skopje</i>		74	3.42
	Skopje	74	3.42
Grand total		1100	1.01

Comparative analysis (field vs. desk research results)

The results show that there are differences in the observed average regional farmland prices resulting from both desk and field research data sources. Table 3 presents these differences in the observed average regional price on agricultural land, which resulted from both data sources, desk and field research. The desk research resulted in higher average price for only two regions, Northeastern and Skopje region. However, these differences are less significant. For all the other regions, the desk research resulted in lower average price for the agricultural land. The most significant difference in the price is observed for the Eastern region, while in Vardar region there is no difference in the registered price between the two different sources of data.

Table 3. Difference in the registered farmland price between two sources of data

Region	Average price (€/m ²) Desk research, 2013	Average price (€/m ²) Field research, 2014	Desk vs. field data (Change +/-)
Eastern	0.39	1.20	-2.08
Northeastern	1.43	1.14	+0.20
Pelagonia	0.47	0.70	-0.49
Polog	2.19	2.40	-0.10
Skopje	3.42	2.80	+0.18
Southeastern	0.51	0.72	-0.41
Southwestern	1.60	2.55	-0.59
Vardar	0.71	0.72	-0.01

The average farmland price resulting from the desk research is around 1 €/m², ranging from 0.39 €/m² observed in the Eastern region to 3.42 €/m² in the Skopje region. On the other hand, the average farmland price resulting from the field survey is 1.53 €/m², ranging from 0.7 €/m² registered in the Pelagonia region to 2.83 €/m² in the Skopje region.

Analysis of the factors affecting the farmland prices

In regards to the factors affecting the farmland price shown in Table 4, density showed the strongest correlation with the average price of agricultural land, and this relationship is positive. This is a logical result since higher density increases the price of the agricultural land, and vice versa. Furthermore, the total arable land per capita is negatively correlated, that is, the higher utilization of arable land results in lower farmland prices, and vice versa. From regional infrastructure, only local road network influence the price of agricultural land, and the more developed is the local road network, the higher the farmland price is. The water supply is only correlated with the price obtained from the field research, which is not a case with the desk research data. However, it is reasonable that the well developed water supply systems should contribute to higher prices of agricultural land.

Table 4. Factors affecting the farmland price (*correlation coefficients*)

Factors Regional data, SSO*, 2011	Average price (€/m ²) Desk research, 2013	Average price (€/m ²) Field research, 2014
Density	0.904917824	0.705985
Arable land per capita	-0.885200297	-0.850600
Population 65+	-0.512331695	-0.625130
Local road network	0.561281472	0.798591
Water supply	0.344917376	0.633251
Installed electricity	0.057582838	0.038760
GDP per capita	0.160425359	-0.093790

*SSO, State Statistical Office, Regional Statistics, 2012

Conclusions

The initial data on the farmland prices in the RM was based on the desk and field research methods. Thus, the average regional prices were determined and compared for existing differences in the prices recorded by the different collection method. The average farmland price resulting from the desk research is around 1 €/m², ranging from 0.39 €/m² observed

in the Eastern region to 3.42 €/m² in the Skopje region. On the other hand, the average farmland price resulting from the field survey is 1.5 €/m², ranging from 0.70 €/m² registered in the Pelagonian region to 2.83 €/m² in the Skopje region. In general, the highest price of a farmland is observed in the Skopje region, while the lowest in the Vardar and the Southeastern region. However, there were great variations in the farmland prices from one municipality to another so the calculated average regional price may not reflect the real regional farmland price.

Additionally, the possible relationship between recorded prices and external factors suggested by the literature were identified by applying correlation statistics. In general, density showed the strongest correlation with the average price of agricultural land, and this relationship is positive. Other important factors that determine the farmland price are the total arable land per capita, the local road and water supply infrastructure.

These initial data and information shall serve as a basis for future development of permanent records on the farmland price data in the country, that are necessary to persuade timely information on the farmland market value and ease the farmland transactions.

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FINANCIAL ANALYSIS OF THE WINERIES IN THE REPUBLIC OF MACEDONIA

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Abstract

The aim of this research is to determine the financial condition of the wineries in the Republic of Macedonia. In these regards, the following standard financial indicators available in the literature are used: 1) Profitability ratios; 2) Debt ratios; 3) Liquidity ratios, and 4) Activity ratios. From the results of the financial analysis it is evident that the wineries are not liquid entities, generally due to the high proportion of inventories in their total assets. However, this is within the nature of the wine sector. Furthermore, during the observed period, the wineries undertake investment activities due to the improved investment conditions to finance investments. In regards to their profitability strategy, wineries increase their asset turnover rather than to pay attention to the value added production of the wines, therefore using low profit margins in the price formation. However, they still succeed to collect claims for a relatively short period of time.

Key words: financial analysis, wine sector, Republic of Macedonia.

Introduction

Agriculture is of great importance for the overall economy in the Republic of Macedonia. It accounts for approximately 10% in the formation of the country's GDP and covers 19% of the active employed population. The share of export of agricultural products is significant and contributes to 9% of the total foreign exchange earnings from exports of goods (MAFWE, 2014). Viticulture, along with wine production, contributes to about 17% - 20% of the agricultural GDP. Wine, after tobacco, is the second most important agricultural product in terms of the export value of the agricultural production. Currently, there are about 90 registered wineries with a total wine processing capacity of 2.338.467 hl. This capacity is approximately two times greater than the annual production of grapes. During the last ten years, there is an increase in the number of wineries (mainly small and medium sized wineries), generally due to the increased production of high quality bottled wines, achieved with control of varieties, vineyards and the harvesting, but also with the improved processing and marketing technologies (MAFWE, 2012).

Capital market deficiencies along with turbulent political, economic and social environment may affect financial stability of the wine sector in the Republic of Macedonia. Previous studies analyze the financial condition of the agricultural sector in the Republic of

Macedonia. For instance, Simonovska *et al.* (2014) observed the financial condition of agricultural companies and econometrically tested their capital structure strategies in increasing profitability, so they defined a typical farm company which is a low-levered farm company showing a positive net sales growth, relying on assets rather than debt, smaller than the average size in terms of net sales, diversifying production with small inventories, operating at high capital intensity and able to cover current liabilities taking advantage of the fluctuations in the business cycle. Typically for the wine sub-sector, Nacka *et al.* (2014) studied marketing strategies of Macedonian wineries in their commitment towards Intellectual property rights, and econometrically tested low-level committed wineries with financial variables and found out that Intellectual property assets were not considered as a strategy for creating competitive market position due to a lack of marketing investments, necessary for developing a strong brand identity.

However, up till now, there is not any research that presents the financial state of Macedonian wineries, which is a starting point in further wine business assessments. Hence, the aim of this study is to determine the financial condition of the wineries in the Republic of Macedonia. The financial analysis performed in this paper, includes analysis of the financial indicators of profitability, liquidity, activity and leverage.

The term analysis in its original meaning suggests examination of a certain condition through its components. Analogously, when it comes to financial analysis, it is an examination of the financial situation of entities through the examination of its constituent elements which reflect the financial condition of these entities. In addition to each of these segments, there is a certain set of indicators by which to obtain answers to the following questions (Arsov, 2008): 1) How effectively, or how actively the entity uses its available assets; 2) Is the entity liquid, or is it able to pay its liabilities; 3) What is the profitability situation of the entity; 4) How much debt the entity has and what is its capability to service the debt; 5) How the market, or the investors, value the company and its financial result, etc.

Financial analysis is a common tool that is used as a standard procedure in the assessment of economic entities' creditworthiness (Altman, 1968) and allows a determination of economic entities' financial position, which is a base for proposal of new investment activities (Smith and Reilly, 1975). The financial ratio analysis provides assessment of the three criteria that determine the economic utility of economic entities, which are, increasing profitability, reducing risk levels and providing liquidity (Barry *et al.*, 2000). By this way, it allows recognition of critical moments that may jeopardize the financial performance, and accordingly indicates if any corrective measures should be undertaken while managing the business so to create basis for better financial performance (profitability) (Simonovska *et al.*, 2014).

Materials and method

The financial determinants are obtained for 16 Macedonian wineries observed during the period from 2008 to 2013. These wineries cover 98.4% of the installed capacity for wine production in the RM. The official records on wineries, in the form of financial reporting statements (balance sheets and income statements) are provided by the Central Register of the Republic of Macedonia (CRM, 2012). The wineries liquidity structure is explained by the current and quick liquidity ratio. Liquidity ratios show the ability of the company to

timely payback its liabilities. If the company is not liquid, it means that its survival is threatened. The difference between the current ratio and the quick liquidity ratio is that the later ratio excludes inventory from current assets, as being low liquid asset. Activity ratios show how wineries efficiently use their assets, or the speed of their assets transformation into cash. The asset turnover is the ratio of net sales over total average assets measured between two time periods. It is a measure of sales income per employed assets. The higher the ratio is, the more efficiently the assets are used by the winery. The opposite is a rule for the average payment period and the days in inventory, or the lesser the period is, the greater the winery's efficiency is.

In order to determine the financial strategy of wineries to increase their opportunities for profit, two indicators on capital structure are considered. The debt-to-equity indicator is a ratio of total debt over total equity. On the other hand, the debt ratio shows the proportion of total assets financed by external sources of capital. Financing through debt is considered to offer the lowest cost of capital.

The wineries profitability structure is explained by the gross and net profit margins and the returns on assets and equity. The gross profit margin calculates the share of operating profit to revenue sales, and the net profit margin represents the share of net profits from revenue sales. These indicators show the level of profit for each unit generated revenues from sales. If the gross is higher from the net profit margin, indicates that non-operating expenditures or the tax rate have increased for the company. Return on assets (ROA), also recognized as to return on investments, measures the rate of return on assets employed by the company and shows how profitably the winery is using its assets. The return on equity (ROE) shows the share of the net profit attributable to equity owners for each unit of capital invested in the company. In favourable credit conditions, the amount of ROE should be greater than ROA. Profitability ratios, ROA and ROE, could be decomposed so to see what the cause of their change is. ROA can be broken down as a product between the net profit margin and the asset turnover so to determine the profitability strategy (is it value-added or volume based). ROE can be broken down as a product between the net profit margin, the asset turnover and the equity multiplier, which is a ratio between the average assets and equity.

The procedure for calculation of these financial indicators is simple, but their interpretation is specific and depends on several factors, especially important is the type of the industry (Helfert, 1997), in this case the wine sub-sector. There are three main types of comparison for assessing the financial indicators: trend analysis, benchmark analysis and cross-industry comparison (Horngren et al., 2010); however, considering the data type and the aim of the study, we only use the first two approaches so to provide evidence on the structural determinants of profitability of the selected Macedonian wineries.

Results and discussion

The results of this study represent the financial situation of the wineries in the country, during a six-year period, from 2008 to 2013. The results are presented subsequently by the four groups of indicators (Table 1 and Figure 1).

The liquidity condition of the wineries is represented by the current and quick liquidity ratio. The current liquidity ratio for the observed wineries is calculated to be 2.05 on average. However, this average value is generally determined from the high current

liquidity of wineries observed during 2010, which is most likely a consequence of the increased wine quantities during the post-crisis period, when the aggregate demand was reduced so the wine was not sold. The great variation in liquidity between wineries means that only some of them are liquid. Therefore, it is difficult to generalize for all wineries and to conclude that the wine sector in the country is liquid. For this reason, the quick liquidity ratio may be more appropriate indicator for wineries liquidity since it excludes inventories from other assets in the calculation of the liquidity. By this indicator, generally wineries are characterized as liquid due to the high average value of 0.82. However, there are significant differences in liquidity of the wineries measured by this indicator as well. With the exception of six wineries, the other 10 are not liquid during the observed period, and this may lead to a bankruptcy and liquidation of these wineries.

Table 1. Summary table for the wineries' financial conditions

Indicator	Benchmark	Average value ($n = 16$)
Current liquidity	$x > 2$	2.05
Quick liquidity	$x > 1$	0.82
Asset turnover		0.41
Receivables turnover		2.88
Average payment period		192
Inventory turnover		1.23
Days in inventory		411
Debt ratio	$x < 0.5$	0.52
Debt-to-equity ratio	$0.5 < x < 1$	1.23
Gross profit margin		0.01
Net profit margin		0.02
Return on assets	$x \approx 0.05$	0.03
Return on equity	$x \approx 0.1$	0.13

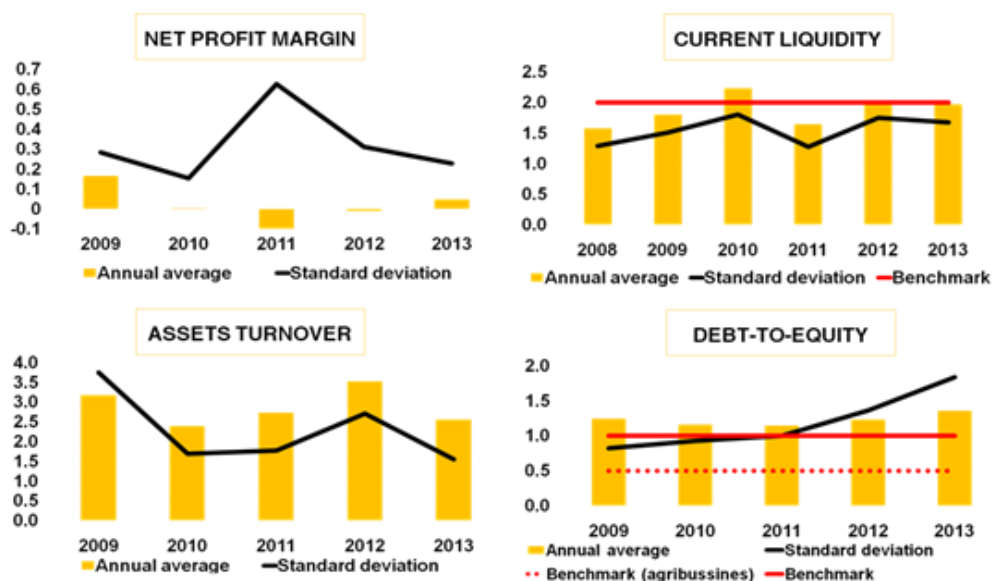


Figure 1. Trends of the financial condition of Macedonian wineries

The activity situation of the wineries is presented through the asset, receivables and inventory turnover, as well as the average payment period and the days in inventory. The average turnover ratio for all wineries in the observed period is 0.41, of which ten wineries are below the sector's average and six have a turnover ratio greater than average. The receivables turnover ratio of the selected wineries is on average 2.88, meaning that those assets that are tied up in receivables are converted into cash 2.88 times a year. The average payment period is relatively good considering the character of the wine production. It ranges from a minimum of 25 days to a maximum of 822 days, which is significantly long. Namely, the average payment period is approximately 4 months.

The inventory turnover ratio for the selected wineries is 1.23, which ranges from 0 to 4.53. Generally, the higher value of the indicator shows a greater efficiency in the use of assets, which is not the case for the sector. If a winery is committed to producing bulk wine, which means cheaper wine, in that case the ratio of inventory turnover will be higher. And vice versa, wineries that are oriented towards producing expensive bottled wines, that are stored for many years due to the technological process, will have a lower coefficient of inventory turnover. However, the most of the wineries have a combined production of a bulk wine and a bottled wine. This is an individual business strategy of the winery and may change from year to year.

The average period for the days in inventory is particularly long, 411 days, but it is within the nature of the wine sector. The large differences in this indicator between wineries underpin the above, which is that their business strategy differs in terms of distribution and the degree of wine differentiation.

The debt condition of the wineries is perceived from the capital structure indicators, which are the debt ratio and the debt-to-equity ratio. The debt ratio for the selected wineries is on average 0.52 and means that the wineries' debt equals their total assets. If this analysis has taken into account the investment activities of the wineries during the analyzed period, then it could be identified why there is high value for this indicator. The debt-to-equity ratio is on average 1.23, which means that for each Denar invested by the owners of the wineries, banks and other lenders are making on 1.23 Denars.

The profitability situation of the wineries is presented by the profit margins and the returns on asset and equity. The average gross profit margin of the observed wineries is 0.01, while the net profit margin is 0.02. The net profit margin represents the share of net profit in sales revenues. The highest value for this indicator is observed in 2013. But, the overall profitability as measured by this indicator is lower than the benchmark of 5% for the other industries. The return on equity on average amounts to 0.13, which means that wineries have 13 Denars profit from 100 Denars invested equity.

Generally, the wineries do not represent liquid companies due to the large share of inventories which is within the nature of the wine sector. Wineries undertake investment activities in the analyzed period, which can be observed through several indicators. For example, borrowed capital exceeds the total assets of wineries that ensures that they are in a phase of investment in capital investments, and return on equity is higher than that of total assets confirms increased investment activity in the wine sector, and improved conditions for investment financing.

Conclusions

From the financial analysis of the 16 selected wineries that cover 98.4% of the installed capacity for wine production in the country, it could be generalized their financial condition. Namely, wineries do not represent liquid companies, generally due to the large share of inventories in their assets which is within the nature of the wine sector. However, wineries manage to collect their receivables in a relatively short period. Furthermore, wineries undertake investment activities in the analyzed period due to the improved conditions for financing investments. For this reason, the use of external capital to finance investment is frequent. In the analyzed period there was a change in the tax policy, as well as changes in the use of non-operating assets, resulting in increased non-operating expenses. Wineries use significantly lower margins in the formation of the price, but large turnover of assets as a strategy to increase profitability. This strategy mainly occurs due to the large production of bulk wine because of the low level of differentiation of the wines in bottles since the purchasing power of domestic consumers is low and since there are difficulties for the wineries to enter foreign markets. Those wineries that work with high margins and added value production have the capacity to absorb economic turbulences through their price flexibility.

Overall, wineries have still not developed a good market-oriented strategy for monitoring the signals of the market, which can be negatively affected with the increased competition in the wine sector if the country joins the European Union.

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UDC: 658.14:005.52]:663.28(497.7)
Original scientific paper

COOPERATION ATTITUDES OF FARMERS IN THE REPUBLIC OF MACEDONIA IN THE PROCESS OF RDP GRANT APPLICATION

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Abstract

The dispersion of information at local level, social capital and cooperation between farmers are very important aspects for institutional growth and development. With the recognized importance of cooperative action and the benefits of the rural development programs for the rural areas and population in transition countries, there is a need for deeper insight into the factors that affect the (dis)incentives for cooperation among the farmers in Macedonia. The study was designed as an exploratory research by employing descriptive statistical analysis and hypotheses testing in order to open potential issues for further research. The results indicate that farmers recognize the benefits of cooperatives and cooperation. The underdeveloped social capital and lack of trust and information are the main factors that influence the cooperation attitudes of farmers in the country.

Key words: cooperation, information, rural development, social capital, trust.

Introduction

Considering the significant rural area and rural population in the Republic of Macedonia, where agriculture is the main source of income, fostering rural development through social capital is very important, especially when the designated budget is not fully utilized.

Social capital is receiving an increasing consideration in debates on rural development as a key factor in developing rural areas (Michellini, 2013). Social capital includes formal or informal social structures of cooperation for mutual benefit that offer a suitable arrangement for better information flow (Bordieu, 1983; Coleman, 1990; Woolcock, 1998). Formal social capital involves participation in formally constituted organisations and activities, while informal social capital involves informal social relations (networks) (Pichler and Wallace, 2007) that shape social life and economic activities (Granoveter, 2005). Grootaert and Bastelaer (2002) identify two forms of social capital. The first is its structural form which includes the observable social structures (networks, associations, and institutions) and the rules they embrace. The second is its cognitive side which contains more abstract and intangible elements such as attitudes and norms of behaviour, shared values, reciprocity, and trust.

Macedonia experiences lower levels of social capital and distrust, especially in the institutionalized forms of cooperation. The main factor for the poor formal cooperation in rural areas is the existing distrust in public institutions since they are influenced by the

state (Lissowska, 2013) as a consequence of the negative past experience with agricultural cooperatives in the former socialist system. There were attempts to revive and introduce the concept of modern cooperatives in the country by donor projects. Regardless, there are still a limited number of cooperatives and other formal organizations that effectively support information flow in rural areas. Many recently introduced cooperatives ceased to exist after the funding period due to their inability to sustain cooperation and development (Nikolic *et al.*, 2015). Since formal networks remain unsuccessful structures for dispersion of information in rural areas, informal networks may present a valuable source of social capital and information exchange. In fact, Fukuyama (1995) and Murray (2006) emphasized that informal social capital is particularly strong and stable in times when the formal structures are weak.

Membership in cooperatives, civic groups or public institutions was evidenced to positively influence growth (Raiser *et al.*, 2002). Since dispersion of information at local level is very important for improving the consumption of grants from the rural development programme, it is necessary for rural population to achieve higher levels of social capital. In this regard, formal and informal cooperative actions in rural areas have a recognized importance. Informal networks may substitute formal networks in cases when formal cooperation is absent or weak. Therefore there is an urgent need to primarily identify the level of social capital and trust in the formal (institutionalized) structures, so to set a basis for identification of the development level of the informal ones. Hence, *the aim* of this study is to identify the reasons behind the (dis)incentives for formal rural networking in the country during the process of application for the rural development programme. This research focuses on farmers, as they represent the majority of rural population in the country where agriculture is a main source of income in rural areas.

In this regard, the study is designed as an exploratory research by employing descriptive statistical analysis and hypotheses testing in the framework of the social capital theory. It is expected the observed results to lay the initial groundwork for future research on this issue for the Republic of Macedonia.

Following the introduction, the subsequent section describes the data and the research method in details. The next section presents the description of the sample. The hypotheses are then set, followed by the results. At the end, short discussion supports the conclusions.

Materials and methods

This study is designed as an exploratory research to determine if the observed reasons behind the (dis)incentives for formal networking among the farmers in the process of grant application for the rural development programme might be explained by the social capital theory.

To address this issue, a survey was conducted in approximately 300 farm households in Macedonia, during November–December 2014. The selection of the sample considered previously defined criteria in terms of: (1) household location and its level of networks development, and (2) rural household type and its perspective future in agriculture. By the first criterion, two pilot regions were selected: the rural area of the municipality of Strumica represents the pilot region with an existing formal network organisation, while the rural area of the municipality of Bitola is the pilot region without

an existing formal network organisation. The second criterion resulted in selection of those rural households that are classified as farm households which are economically and demographically viable. These are farm households with at least two members, out of which at least one is younger than 50 years of age.

The survey followed a structured questionnaire, including: (1) socio-economic characteristics of farmers, and (2) farmers' opinions on reasons behind the (non)membership in a particular network (formal or informal), in the context of grant application for the rural development programme. The first part of the questionnaire covered background information on: a) farmer and household profile (i.e., age, gender, education, primary occupation, and main household income), b) farm type (production orientation) and size, and c) farm performance (i.e., farm profitability assessment, dependency on subsidies to break-even, intention to invest and the type of investment, expectancy to continue farming in the next 3–5 years, and availability of a farm successor). The second part of the questionnaire covered questions focused on farmers' memberships in organisations and if not being a member, their reasons for avoiding membership. In fact, the first part of the questionnaire describes the sample by using descriptive statistics, while the second part targets the prime aim of the research through hypothesis testing.

In particular, alternative hypotheses include reasons behind the disincentive to be a member of a cooperative between cooperative members and non-members, such as lack of trust, time or information, as well as reasons such as farmers not seeing benefit in cooperation actions, disliking cooperative members, considering that cooperatives do not function well or thinking that there are no such organisations. These hypotheses are tested by applying nonparametric chi-square test in Stata 12.0 (StataCorp, 2011) since data are nominal. The chi-square test is used to determine whether there is significant difference between the empirical (observed) frequencies and the theoretical (expected) frequencies of the sample modalities (Risteski and Tevdovski, 2008).

Description of the sample

The exploratory research examined two regions, the rural areas of the Strumica and Bitola regions. In the Strumica region there is an existing and functional cooperative, while in the Bitola region there is no existing formal network organisation. The description of the sample is summarized in Tables 1 to 4.

The majority of respondents in the observed rural areas are male, in average 47 years of age, with a secondary school of education (Table 1). Agriculture is the primary occupation of the majority of heads of the household with no other employment out of agriculture. In fact, 90% of the household income comes from agriculture, mainly from a labour intensive production. The majority of farmers have long experience in farming.

Table 1. Summary statistics for the sample profile in selected rural areas

<i>Farmer and household profile</i>	Country level
Age (mean $\pm$ standard deviation; median)	46.5 $\pm$ 12.2; 45
Male respondents (%)	92
Level of education (%)	
Primary education (4 years)	8
Primary education (8 years)	36
Secondary school (3-4 years)	49
College (2 years)	2
University (4 years)	4
Agriculture as household primary occupation (%)	95
Household income from farming (%)	90
Years in farming (mean $\pm$ standard deviation; median)	25.4 $\pm$ 11.6; 25

The main production orientation in the rural municipalities of Bitola is dairy farming with an average herd size of 11 heads, while in the rural municipalities of Strumica it is early-vegetable production with an average farm size of 0.7 ha (Table 2).

Table 2. Summary statistics for the farm type and size (mean $\pm$ standard deviation; median)

<i>Farm type</i>	<i>Farm size</i>	
	Strumica region	Bitola region
Farmed area (ha)	1.2 $\pm$ 0.7; 1	9.4 $\pm$ 18.7; 5
Cereals	0.3 $\pm$ 0.4; 0.2	4.1 $\pm$ 7.4; 2
Industrial crops	/	2.6 $\pm$ 5.6; 1
Fodder crops	0.1 $\pm$ 0.2; 0	2.1 $\pm$ 7.7; 1
Vegetables	0.7 $\pm$ 0.4; 0.7	0.3 $\pm$ 1.0; 0
Orchards	0.0 $\pm$ 0.1; 0	0.0 $\pm$ 0.2; 0
Vineyards	0.0 $\pm$ 0.1; 0	0.0 $\pm$ 0.2; 0
Total livestock units	0.3 $\pm$ 0.9; 0	12.1 $\pm$ 26.2; 6
Cattle	0.0 $\pm$ 0.2; 0	10.7 $\pm$ 26.4; 5
Pigs	0.3 $\pm$ 0.8; 0	0.6 $\pm$ 2.2; 0
Sheep	1.6 $\pm$ 7.2; 0	9.2 $\pm$ 38.2; 0
Poultry	0.2 $\pm$ 1.7; 0	8.6 $\pm$ 33.6; 0

Large part of farmers was unable to evaluate the performance of their household in relation to the others; however a slight optimism exists among them in regards to their own farm performances. Large percentage of farms perceived to be dependent on subsidies to break-even, though there is significant regional difference regarding this issue. For instance, majority of vegetable farms from the Strumica region stated that are not dependent on subsidies to break-even(since vegetable production is profitable), while those in Bitola region are very dependent on them because of the unfavourable farm structure and the poor governance structures in dairying.

Half of the surveyed farmers plan to invest in a mid-term perspective, mainly in extension of the current production and in equipment. There still is a lack of incentives among them to apply for the available governmental support, even though on-farm investment support dominates in the structure of the rural development program (Volk *et al.*, 2014).

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Table 3. Summary statistics for the farm performances

<i>Farm performance measures</i>	Country level
Perceived performance relative to others (mean $\pm$ standard deviation; median)	3.3 $\pm$ 0.9; 4
Dependency on subsidy to break-even (%)	
Not dependent	41.2
Slightly dependent	21.8
Very dependent	37.0
Plan to invest in the next 3-5 years (%)	
Definitely not	13.5
Unlikely	13.1
Not sure	16.2
Very likely	25.9
Definitely yes	31.3
Planned type of investments (% yes)	
Equipment's	22.6
Equipment	44.8
Land	4.4
Extension of production	47.1
Diversification	0.7
Rural tourism	0.3
Livestock	15.2
Other	5.7

One reason for the low utilization of the budget of the rural development program is the poor setting of the governance structures for implementation of policy reforms. These governance structures should include wide array of agents and organizations, such as farmers, households, consumers, governmental and non-governmental organizations, etc.

In order to improve the access to information and achieve growth of their farm economy, farmers should be encouraged to participate in formalized voluntary organizations (Koutsou *et al.* 2014). Nevertheless, the participation in formally organized networks in the regions subject to this research is very low. Only 7% of the surveyed farmers are members of a cooperative, out of which 14% are from the Strumica region. In the Bitola region, there are no members of a cooperative since there is no existent cooperative, while in Strumica there is a cooperative that operates well. Another detected form of formal networks in the observed rural areas was the membership in non-governmental organizations (NGO), such as producer/farmer organizations and associations. These usually provide logistical support to farmers so their activities are primary oriented towards representation, lobbying, and advocating for farmers' interests; advising, informing, and training of farmers.

Table 4. Summary statistics for formal networking among farmers

Region	Member in a formal network	
	Cooperative (%)	NGO (%)
Country level (n=298)	7.0	3.0
Strumica (n=150)	14.0	1.3
Bitola (n=148)	0	4.7

From the results, it is evident that NGOs work better in regions where there is no active cooperative, but the farmers' interest to be a part of such a network is still low. However, this may indicate that, on one hand, there is a need farmers' to be supported by formal networks, but on the other hand, farmers do not show great interest to be a part of them due to still not enough explored benefits. This observation lays groundwork to further explore the disincentives among farmers to be active members in formal organizations.

Hypotheses setting

Several hypotheses are proposed to identify the (dis)incentives among farmers for (not) being members of cooperatives in the grant application process for rural development programme. In fact, we test if there is significant difference in the expressed opinions between members and non-members of cooperative in regard to several reasons behind the disincentives to belong to any formal organisational arrangement, such as lack of trust and lack of information. Members and non-members of cooperative may express different opinions on this issue since they have different experiences, i.e. members may conceptualize on a past experience, and non-members do not have any experience but just beliefs. Explanation behind the setting of each hypothesis follows below.

In socialist economies, cooperative type of organisations were often non-voluntary and governmentally imposed (Raiser *et al.*, 2002). The misinterpretation of the current meaning of cooperatives created distrust among farmers for the real purpose behind their existence, which is to economically support farmers' interests. Since social capital is related to the local conditions and the historical background of the society (Productivity commission, 2003), it is assumed that trust (lack of trust), as the most important factor of social capital, is the main reason for farmers to avoid membership in formal organizations, and therefore sets the first hypothesis.

Hypothesis 1: *There is significant difference between members and non-members of cooperative in their opinion that lack of trust is an important reason for not being a member of a formal organisational arrangement.*

Furthermore, because of the presumption of low levels of social capital in post-socialist countries, farmers' formal and informal networks are expected to be underdeveloped. Social networks are important source of information and opportunity possibilities (Traikova *et al.*, 2008), and their underdevelopment can often be a limiting factor in the case of information transfer for the existence of cooperative type of organization and their role. This sets ground for the second hypothesis.

Hypothesis 2: *There is significant difference between members and non-members of cooperative in their opinion that lack of information is an important reason for not being a member of a formal organisational arrangement.*

Several sub-hypotheses are proposed in order to obtain deeper understanding of the main reasons for the low participation and farmers attitudes towards membership in formal networks (outlined in the two preceding hypotheses).

Sub-hypothesis 1: *There is significant difference between members and non-members of cooperative in their opinion that farmers think there are no organizations of that type is an important reason for not being a member of a formal organisational arrangement.*

Sub-hypothesis 2: *There is significant difference between members and non-members of cooperative in their opinion that farmers do not see a benefit from the*

membership is an important reason for not being a member of a formal organisational arrangement.

Sub-hypothesis 3: *There is significant difference between members and non-members of cooperative in their opinion that farmers do not like the people from the cooperative is an important reason for not being a member of a formal organisational arrangement.*

Sub-hypothesis 4: *There is significant difference between members and non-members of cooperative in their opinion that cooperatives do not function well is an important reason for not being a member of a formal organisational arrangement.*

In this regard, lack of information to farmers could lead to farmers' inability to recognize the existence of cooperatives as well as the benefits stemming from membership in cooperatives. Assumingly, the lack of trust creates aversion towards other cooperative members and their intentions, therefore farmers are expected to perceive cooperatives as non-functional organizational arrangements.

Status of formal cooperation in rural areas

According to the summary results from the survey (Table 5), farmers did not show high interest in expressing their reasons for low participation in formal organisational arrangements. This may indicate that farmers do not believe in formal networks and the interest for their membership in such organisations is low. These facts go back to the main assumed reasons for the existence of the low level of social capital in rural areas, which are the lack of trust and information.

Table 5. (Dis)incentives for formal networking among farmers

Reasons for not being a member of formal network	Yes (%)
Lack of trust	20.5
Lack of information	12.4
Farmers do not see a benefit from the membership	7.7
Farmers do not like the people there	1.0
Farmers perceive that they do not function well	3.7
Farmers think there are no such formal organizations	35.2

However, the observed attitudes of farmers towards membership of organisations create a different image (Table 6). In fact, optimism exists among farmers in their perception of the benefits from the formal cooperative actions which may be an indicator for future motives of farmers to become part of some kind of a formal network. Majority of the farmers agree that membership in organizations is useful (over 70% of respondents). They also agree that organizations contribute to the development of the village and disagree that the members of the organisation only think of themselves and their interest and that they respect joint agreements.

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Table 6. Farmers' perception of formal networks

<i>Personal attitudes of farmers towards membership of organisations</i>	In %
Membership in organization is useful	
Strongly disagree	2.3
Disagree	9.0
Don't know	16.7
Agree	35.5
Strongly agree	36.5
Organizations contribute to development of the village	
Strongly disagree	2.3
Disagree	8.4
Don't know	22.1
Agree	40.5
Strongly agree	26.8
People of organizations only think of themselves and their interest	
Strongly disagree	17.7
Disagree	17.7
Don't know	26.8
Agree	27.8
Strongly agree	10.0
Farmer believes that members in organization respect joint agreements	
Strongly disagree	7.0
Disagree	12.0
Don't know	38.8
Agree	26.4
Strongly agree	15.7

Table 7. Farmers' intentions towards formal networking

<i>Intention of farmers towards membership in organisations</i>	In %
Farmers' assessment of their intention to become a member of organization for their own benefit (to get information, technical support, advise, experience, etc.)	
Very weak benefit	12.1
Weak benefit	13.8
Don't know	53.7
Strong benefit	13.7
Very strong benefit	6.7
Farmers informally cooperate between each other	
Yes	93.9
No	6.1
Frequency of informal cooperation	
Never	2.7
Rarely	13.2
Not sure	9.2
Sometimes	48.8
Always	26.1
Reasons for informal cooperation (% yes)	
Information exchange	19.6
Technical support	14.5
Common problems	45.3
Informal socialization	36.5
Other reasons	32.1

In this regard, through assessment of their intention to become a member of formal organisations (Table 7), it is found out that farmers are mainly indifferent since they are not aware of the benefits from membership. It is easier for them to create social ties on informal than on formal level. Hence, agricultural producers mainly informally cooperate between each other on occasional basis. The main reasons for this cooperation is sharing common problems and socializing between each other.

Since rural development programme is aimed to foster development of rural areas, farmers were assessed if they are aware of the benefits from this programme and if they consider that it may lead to higher networking of rural population (Table 8). Farmers show some level of awareness for the benefits of the rural development programme, especially for that it supports the survival of family farms. This is important for economies in transition; however, the policy has to be thorough to prevent negative effects from the imposed support. Negative effects may result in hindering the development of the agricultural sector as farmers may not be motivated to restructure. In regard to the rural development programme as a mediator in achieving higher levels of informal social capital, farmers are neutral. However, there is a slight positivism among farmers which think that this programme is a good way to communicate between each other.

Table 8. Farmers' awareness for the benefits of the rural development programme

<i>Awareness of farmers for the RDP benefits</i>	In %
RDP measures support the survival of family farms	
Strongly disagree	1.7
Disagree	3.3
Don't know	21.1
Agree	38.1
Strongly agree	35.8
RDP leads to higher networking of rural population	
Strongly disagree	2.0
Disagree	10.4
Don't know	45.8
Agree	29.8
Strongly agree	12.0

In order to explore the reasons for the low interest among farmers to be a part of formal networks, several hypotheses were tested. Moreover, a chi-square test of independence was performed to examine if there is significant difference in the expressed opinions between members and non-members of cooperative in regard to several reasons behind the disincentives for the membership in a formal network (Table 9).

The results from the tests show that the lack of trust is a major disincentive factor for farmers not to be a member of a formal network, but this observation differs by the current membership status, $\chi^2 (1, N = 298) = 5.81, p = .02$. In fact, those few members of a cooperative think that lack of trust should not discourage other farmers to become members of formal arrangements. Lack of information, on the other side, is not a strong disincentive factor for farmers to not participate in a formal network and this perception does not differ by their current membership status, $\chi^2 (1, N = 298) = 3.20, p = .07$.

Even though formal networking among farmers is not hindered by the lack of information, the largest part of farmers are still not aware of the existence of such formal

organisations that aim to support their interests. However, this responsiveness differs by the current membership status, $\chi^2 (1, N = 298) = 12.29, p = .00$, which is logical since those that are members of organisation are aware that formal networks exist unlike the non-members. Although farmers do not believe that lack of information discourages farmers for formal networking, it could still lead to farmers' inability to recognize the existence of cooperatives. In addition, farmers do not perceive that formal networks are not beneficial for them and this perception does not differ by their current membership status, $\chi^2 (1, N = 298) = 1.89, p = .17$. Also, members and non-members of cooperative do not recognize that the aversion towards other members of formal networks should stop farmers to become a part of it, $\chi^2 (1, N = 298) = 0.23, p = .63$. Moreover, farmers do not believe that the perception of cooperatives as non-functional formal arrangements is among the farmers' disincentives for formal networking, and this perception is shared by the members and non-members of cooperative, $\chi^2 (1, N = 298) = 0.87, p = .35$.

Table 9. Hypotheses test results with a membership of cooperative as a dependent variable

Hypothesis	Independent variable (Discouragements to be a part of a formal network)	Rejected null hypothesis (+); otherwise (-)
H1	Lack of trust	+
H2	Lack of information	-
SH1	There are no organisations	+
SH2	Farmers do not see a benefit	-
SH3	Farmers do not like the people there	-
SH4	Farmers think it not function well	-

To sum up, the main disincentives for farmers to become a part of a formal network are the lack of trust in these governance structures and the perception that there are no such formal arrangements. However, those farmers who had an experience with the membership in cooperative have different perception. They believe that cooperatives are based on mutual trust to support their interest. The positive attitudes towards formal networks are a good foundation for promotion of cooperative type of organisations. However, much has to be done so to raise the trust and thus the awareness for the benefits of the formal networking.

Discussion and conclusions

Based on the socialistic background, the Republic of Macedonia is expected to experience lower levels of social capital and distrust in the institutionalized forms of cooperation. Trust in institutionalized type of organizations was destroyed during the socialistic period. This was reflected in the small number of functional agricultural cooperatives and organizations, with low importance and power to influence farmers' position in the agricultural value chains. Additionally, there is a low horizontal and vertical integration, and absence or low efficiency of institutions for logistic support, all of which are considered to be among the main obstacles for better consumption of the rural development funds (Bogdanov *et al.*, 2015).

Farmers in the rural areas of the observed regions, although small and highly dependent on agricultural incomes and subsidies to break-even, still avoid membership in

cooperatives which can provide them with access to information and resources. The dispersion of information at local level is crucial for improving the consumption of grants from the rural development programmes. However, farmers often have limited access to information on the available rural development programmes as well as the possibilities these programmes provide for their individual (farm) and rural development.

Even after two decades of transition in the Western Balkan countries, the governance structures for policy reforms implementation are still poorly developed and this is reflecting on the development of the rural social networks which are also weak. As expected, farmers avoid participating in cooperatives mainly due to the lingering distrust in any institutionalized or governmentally induced cooperation. On the other hand, farmers' which experienced cooperative collaboration through their membership in certain formal networks, expressed trust that other farmers, which are cooperative members, would not act opportunistically and only in accordance to their own interests. Additionally, farmers show high consciousness for the usefulness of membership in organizations as well as the possible contribution of such organization for the development of their villages. This positive personal perception of cooperation provides a positive sign for the farmers' intentions to become part of certain formal network in the future, providing that they will be informed for all of the existing organizations that might be of assistance for them to pursue their interests. At the moment, many of the farmers are unaware for the existence of cooperatives or similar type of formal network in their immediate surrounding or even on a broader level.

The fact that farmers still associate the old concept of cooperatives which existed in the former socialist system leads to constant lack of trust and confidence among farmers in the rural areas, and this is a major factor and obstacle for the development of social capital in these areas. This inherited distrust is largely linked to the lack of information and opportunity for farmers to acquire knowledge and experience regarding the modern forms of cooperative association. This is why it is important that the level of informal social capital among the farmers is included and examined in future research. This is especially important in order to evaluate farmers' mutual trust and willingness to cooperate. The mapping of these informal network structures may serve as a base for conceptualization of the future institutionalized models of cooperation in agriculture. In addition to the identified farmers' views and attitudes, the models will be adapted and suitable to fit the specific social capital structure and institutional setting in the country.

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UDC: 332.14(497.7)
Original scientific paper

BEHAVIORAL FACTORS INFLUENCING THE USE OF RURAL DEVELOPMENT SUPPORT

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Abstract

Rural development (RD) policy is essential for sustainable development and continuous improvement of the quality of life of rural people. Despite the relative importance of RD policy, its implementation is lagging behind the direct payments support, partly because of the farmers' unengaged interest. The aim of this paper is to identify the key behavioral factors that influence farmers' intention to apply for RD support. The analytical framework is based on the theory of planned behavior, where the individual intention to perform a given behavior (apply for RD support) determines the performance and is shaped by the individual attitudes, social support and the perceived behavioral control; and a structural equation model. The survey took place in November-December 2014, in face-to-face interviews covering 299 farm households in Macedonia. Farmers with more positive attitude and perceived behavioral controls have stronger intention to use the RD support available. The model explains two-thirds of the variance. This knowledge provides basis for designing interventions to maximize farmers' use of available rural development funds.

Key words: Rural development support, Macedonia, Theory of planned behavior, Structural Equation Model

Introduction

The ongoing trend of abandoning rural areas and insufficient use of the land resources is a challenge for the policy makers. Rural development (RD) policy strives to solve these and other rural areas' issues, such as agricultural competitiveness, diversification of employment opportunities, and thus mitigate the poverty and improve the quality of life in rural areas.

Rural areas accommodate nearly half of the population in Macedonia (43% in 2010-2014, WBDA, www). The unfavorable educational structure, poor qualifications and lack of skills of farmers, as core constituent of the rural economy, limit the entrepreneurial potential in the rural areas. The rural economy growth is also constrained by insufficient investments in public goods and services, weak physical infrastructure, limited access to markets and to sources of finance, undeveloped information systems, knowledge and technology transfer. The development of rural areas is additionally hindered by.

Agriculture is the key contributor to rural economy and source of employment in rural areas. The share of agriculture, forestry and fishing in national gross value added is

around ten percent, whereas employment in this sector is 17 percent of total employment in Macedonia (WBDb, [www](http://www.wbdb.org)). Small scale and fragmented family farming is typical, with an average farm size of 1.9 hectares (SSO, 2014). The sector shows low factor productivity, mostly due to slow process of farm consolidation, inefficient use of production factors, and slow restructuring of other sectors. It serves as social buffer for the unemployed population that find shelter within family holdings and semi-subsistence agriculture.

Macedonia aspires to join the European Union, and as candidate country since 2005 is committed to reforms and necessary adjustments towards the Common Agricultural Policy (CAP). The rural development policy is harmonized with the CAP, adopting its conceptual, administrative and financial model. However, agricultural policy is still oriented toward production support, while structural and rural development support receives lower priority. The total budgetary support in agriculture and rural development is very low (148 Euro/ha UAA in Macedonia, one third compared to the support in EU-27) (Volk, Rednak, and Erjavec, 2014); whereas the funds for structural and rural development measures are at much lower level (25 Euro/ha or comparatively at about 10%-level of the EU average) (Dimitrievski et al. 2014). In terms of implementation, among the structural and rural development measures dominate the investment support measures for improving the competitiveness of agriculture. The other measures, such as those supporting agri-environmental issues and rural economy, receive lower attention. Additional support is available through the EU pre-accession instrument for rural development (IPARD), but an extremely low rate of absorption is evident in the previous years (16.3%) (IPARD Monitoring Committee, 2015). The low and unstable level of budgetary support insufficiently addresses most issues hampering rural development (technological backwardness, rural poverty, environmental problems and unfavorable social structures in rural areas).

Despite the relative importance of RD policy, its implementation is far behind the direct payments support, partly because of the farmers' interest for this support. The level of preparedness of farmers affects the rate of utilization of RD support. Farmers face obstacles in accessing these funds, partly from the individual lack of abilities and motivation to deal with the complex rules and procedures, emphasized by existing weaknesses in administrative and institutional capacities. The rural development issues, the policy challenges and the absorption capacity of farmers as main beneficiaries, prompt the need to understand and explain the factors which influence farmers' decisions on rural development support use. In this respect, the aim of this paper is to identify the key behavioral factors that influence farmers' intention to apply for RD support. The behavior of interest in this research is an application to RD support for personal benefit (own household) or public benefit (infrastructural improvements, renewal of the villages, rural tourism etc.) in short- (next year) and mid-term (next 3-5 years) perspective.

The behavioral approach is often used in agricultural and rural studies investigating farmers' response to policy initiatives (Burton, 2004). Besides profit maximization, farmers' behaviors are also influenced by a range of socio-economic and psychological factors (extensive list provided in Willock et al., 1999). Understanding the motivation of farmers, given adequate information on resources and constraints, fairly explains and predicts farmers' behavior (Gasson, 1973). The theory of planned behavior (TPB) (Ajzen, 1991) is the most common model for understanding, predicting, and possibly changing human behavior (Webb and Sheeren, 2006). This theory is highly applicable to agricultural

research (Jackson et al., 2006) and has been applied in studies related to farmers' behavior in agricultural policy context (Garforth and Rehman, 2006; Gorton et al., 2008; Dos Santos et al., 2010; Emery and Franks, 2012) and farm decision-making and management (Willock et al., 1999; Bergevoet et al., 2004; Fielding et al., 2005; Artikov et al., 2006).

TPB suggests that the behavior is guided by the intention to perform the behavior in question, i.e. intentions indicate the degree of effort the individual is willing or planning to put forth in order to perform the behavior. TPB assumes three independent predictors of intention (Ajzen, 1985, 1991, 2012): (1) the attitude toward the behavior as its' positive or negative evaluation; (2) the subjective norm in terms of the perceived social pressure whether to perform the behavior; (3) the perceived behavioral control or the individual assessment of the level of complexity to perform the behavior. The more positive the attitude, subjective norm and perceived behavioral control, the stronger is the intention to engage in a behavior, and the greater the likelihood of the performance of the behavior (Ajzen, 1991). Given the availability and accessibility of the required opportunities and resources, as well as the intention to perform the behavior, the individual should eventually succeed in performing the behavior.

To fulfill the research aim, a structural equation model is built using data collected in a survey in Macedonia, carried out in November-December 2014. The research was carried out in the framework of the regional research project "The impact of socio-economic structure of rural population on success of rural development policy". The findings provide basis for recommendations for policy makers, extension agents and advisors to improve their approach when addressing the farmers and stimulate their participation in RD programs.

After the introduction, we present the methodological framework and data collection procedure. The results of the survey along with the measurement and structural equation model are then presented. The discussion and concluding remarks are provided at the end.

Materials and methods

The questionnaire was designed to obtain measures of the TPB constructs. Eliciting accessible beliefs was done first by using open-ended questions to agricultural experts, supplemented with modal accessible beliefs from the literature review, which were additionally simplified after testing the questionnaire with farmers. The statements are assessed on a 5-point disagree-agree scale, or a Likert-scale given in semantic differentiate format, ranging from 1 (worst evaluation) to 5 (best evaluation).

The survey took place in November-December 2014 in face-to-face interviews in two regions in Macedonia, Strumica and Bitola, ultimately gathering 299 filled questionnaires. During the survey, 46 farmers declined to participate in the survey due to various reasons ("I'm busy", "I don't have time for surveys", "I do not want to share personal information", "I do not want to have problems with the ministry later", etc.). The few missing values in the dataset were imputed using the maximum likelihood method with expectation-maximization (EM) algorithm, suggested as an advantageous approach to traditional techniques (Baraldi and Enders, 2010).

Following the descriptive statistics to profile the sample and the general response, a structural equation modeling (SEM) approach was used to analyze the farmer's intention to apply for RD support.

SEM is a robust analytical tool for studying complex relationships as it combines two multivariate techniques (factor analysis and multiple regression analysis). Covariance matrices are used to assess the model fit hence providing flexibility and relatively greater information content (Hair et al., 2006). Two-step SEM modeling procedure is carried out by specifying first the measurement model (using SPSS, version 20) and then the structural model (using AMOS, version 21). The measurement model, as confirmatory factor analysis representing how the measured variables come together to form the latent constructs, serves as a basis for assessing the fit and validity of the structural theory. In the structural model, representing how the constructs are associated with each other, the analysis focuses on the hypothesized relationships between the latent constructs.

Figure 1 illustrates the relationships of the factor, following TPB: positive attitudes, subjective norms, and perceived behavioral controls enhance the behavioral intention of using RD support. In our study, we delimit the analysis to the point of explaining the behavioral intention to apply to RD support, since strong intention to engage in certain behavior is generally a strong indicator of its ultimate performance (as reported in Sheeran's (2002) meta-analysis, the weighted average correlation of the intention-behavior relationship is 0.53).

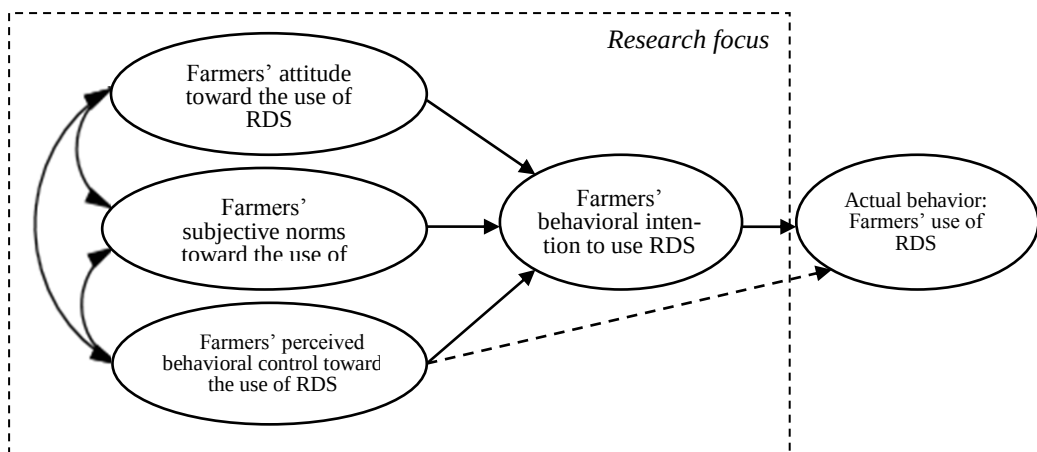


Figure 1. Theoretical concept of the interrelations between behavioral factors and intention (adapted from Ajzen, 1991)

In the model specification, ten variables were retained to explain the interrelations between the farmer's behavioral determinants: two intentions, three statements measuring farmers' attitudes, two subjective norms and three perceived behavioral control (as shown in Table 1).

In the specified model, the behavioral determinants (ATT, SN and PBC) are set as exogenous multi-item latent constructs, while the behavioral intention to apply to RD support (BI) is the endogenous multi-item latent construct variable. Correlation is allowed between the three separate elements of farmer's behavior, given that the inter-construct relationships can increase the reliability of the dependence relationship estimates (Hair et al., 2006).

Results

Farmer's profile

Most of the farmers are with low level of education (in average 10 years of formal education), but with long experience in farming (see Table 1 for detailed sample profile). The average age of respondents is 47, and the average experience in farming is 26 years. Over 90% of the household income comes from farming, indicating that farmers depend almost exclusively on agriculture. Most of the farmers are commercially oriented (the respondents reported that 96% of the production is sold on market). Although expressing very strong intention to stay in farming and in the rural areas (mean 4.6, towards strongly agree), most farmers do not know who will take over their farm and continue the farming activity. Macedonian farmers are familiar with RD support (mean 4.3); around 25% of the respondents applied for some kind of RD support with 65% success rate and average amount of 5,600 Euros per farmer.

The general attitude toward RD support is positive; over 90% of farmers declare that it is good for the state to have RD support (mean 4.6). Farmers express generally positive intention to apply for RD support both in short-term and mid-term perspective (mean 3.4 and 3.5, respectively, see Tab. 1). Farmers found that the principle of co-finance in RD projects is good motivator for farmers (mean 3.7). Macedonian farmers value the approval from family to use RD support (mean 4.1). The opinions of other people they respect are also influential, but expectedly to somewhat lesser extent (mean 3.6). The response on the statement whether many people they know pursues them to apply for RD support calls is rather neutral (mean 3.2). The personal ability of the farmer is generally assessed as an obstacle. Macedonian farmer's perception of lack of experience and knowledge to independently prepare the application has been confirmed in previous research (Kotevska et al., 2013). The access and cost of RD support application (information, procedure and documents) is perceived as an additional external barrier. Regarding the personal ability, they are not self-confident (mean 2.1), and responded mostly negative to the statement whether they have enough own means to co-finance an RDP investment.

Table 1. Profile of the farmer's sample, RD support and TPB statements (mean $\pm$ st.dev)

<i>Sample characteristics</i>	
Number of farmers in the sample	299
Age (years)	46.45 $\pm$ 12.23
Work in farming (years)	25.56 $\pm$ 12.37
Formal education (years)	10.10 $\pm$ 2.86
Share of production sold on market (%)	96.31
Share of income coming from farming (%)	90.35
Likelihood to stay in farming (S1)	4.56 $\pm$ 0.84
Identified successor (S1)	2.92 $\pm$ 1.45
Intention for investment (S2)	3.48 $\pm$ 1.40
<i>RD support</i>	
Familiarity with RD support (S3)	4.33 $\pm$ 0.73
Farmers' opinion: it is good state has a RD support (S3)	4.56 $\pm$ 0.60
Farmers that applied for RD support (%)	25.08
Farmers that received RD support (%)	16.39
Success rate (applied/received, %)	65.33
Received RD support per farmer (Euros)	5,63 $\pm$ 2,50
<i>Farmers' intention to use RD support</i>	
BI1: Intention to apply for RD next calls (S3)	3.44 $\pm$ 1.13
BI2: Intention to use RD for own household next 3-5 yrs (S2)	3.46 $\pm$ 1.09
<i>Farmers' attitude</i>	
ATT1: RD increases income of farms and rural households (S3)	3.98 $\pm$ 0.91
ATT2: RD supports survival of small family farms (S3)	4.03 $\pm$ 0.92
ATT3: The co-financing in RD projects is good motivator for farmers (S3)	3.73 $\pm$ 1.10
<i>Farmers' subjective norm</i>	
SN1: Other people approve application for RDS (S3)	3.59 $\pm$ 0.99
SN2: Many people I know pursues me to apply for the RDP call (S3)	3.18 $\pm$ 1.15
<i>Farmers' perceived behavioral control</i>	
PBC1: Enough information to apply (S3)	2.18 $\pm$ 1.02
PBC2: Knowledge/experience to prepare application (S3)	2.07 $\pm$ 1.04
PBC3: I have enough own means to co-finance an RDP investment (S3)	2.62 $\pm$ 1.27

Scales: S1 (1-definitely not; 5-definitely yes), S2 (1-very weak; 5-very strong), S3 (1-strongly disagree; 5-strongly agree).

The measurement model

The measurement model specified herewith is based on TPB and distinguishes four groups of latent factors: attitude toward the behavior (ATT), subjective norm (SN), perceived behavioral control (PBC) and behavioral intention (BI) (Table 1). In the confirmatory factor analysis, the constructs are assumed as correlated, but not dependent upon another. The measurement model fits and displays overall construct validity, which facilitates the assessment of the connection between the theoretical latent construct and the measurable observed variables i.e. the measures consistently represent the same latent factor. The factor loadings are statistically significant and standardized loading estimates are higher than the minimum threshold of 0.5 in all cases. As shown in Tab. 2, the composite reliability (CR) indicator has values of 0.7 or higher, suggesting good reliability, and the average variance explained (AVE) is over the recommended value of 0.5, suggesting adequate convergence since more than half of the variance is explained by the latent factor

structure (as recommended in Hair et al., 2006). The discriminant validity is also acceptable, since the observed variables fit best in the given parent latent construct. In all cases, the variance extracted score is greater than the squared correlation coefficients among the variables and less than one the absolute value of the correlations with another factor.

Table 2. Indicators of internal consistency and validity (n = 295)

	CR	AVE	MSV	ASV	SN	PBC	ATT	BI
SN	0.843	0.734	0.158	0.070	0.857			
PBC	0.832	0.629	0.040	0.025	0.200	0.793		
ATT	0.769	0.528	0.516	0.233	0.398	-0.160	0.727	
BI	0.806	0.676	0.516	0.180	0.113	0.102	0.718	0.822

Note: CR (Composite reliability), AVE (Average Variance Extracted), MSV (Maximum Shared Variance), ASV (Average Shared Variance).

The structural model

Structural equation model is build upon the measurement model in order to verify the impact of behavioral factors on the farmers' intention to use RD support (see Fig. 2). In the specified structural model, based on TPB, the behavioral determinants (ATT, SN and PBC) are set as exogenous constructs and the behavioral intention to apply to RD support (BI) is the endogenous construct. The correlation is freed between ATT, SN and PBC. Following the modification indices, we further opened the correlation among ATT1, ATT2 and ATT3, respectively and also between PBC1 and PBC2.

The fitness of the model was tested and the model is acceptable based on standard model-fit indicators. The overall model chi-square (χ^2) is with value of 54 and 26 degrees of freedom, i.e. the χ^2/df ratio is 2.08. It is however statistically significant, but this is expected since this statistical test and p-value is less meaningful as sample sizes become larger (Hair et al., 2006). Therefore, other supplementary fit statistics were run and all indicated good model fit (as suggested in Hu and Bentler, 1999): comparative fit index = 0.98; population root mean square error of approximation = 0.06, with two-sided 90% confidence interval ranging between 0.04 and 0.08; standardized root mean residual of 0.06.

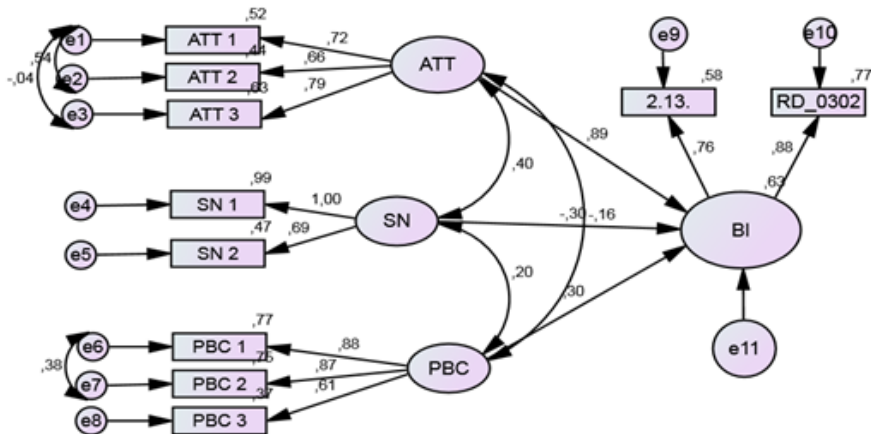


Figure 2. Structural equation model, standardized estimates

The SEM is illustrated in Figure 2; the paths in the model corresponding to the TPB framework. All paths and relations are statistically significant. The more positive attitude and perceptive behavioral control, the stronger behavioral intention to use the available RD support. These three exogenous constructs explain approximately two-thirds of the variance on behavioral intention (squared multiple correlation of the endogenous latent behavioral intention construct: 0.63). The impact of the farmers' attitude whether to apply for RD support has highly positive standardized coefficient (ATT to BI path standardized estimates: 0.886, p-value 0.000). The impact of perceived behavioral control is statistically significant hence confirming that it positively influences the intention to apply (PBC to BI path standardized estimates: 0.304, p-value 0.000). The subjective norms are statistically significantly influencing the intention to apply, but in a negative direction (SN to BI path standardized estimates: MK -0.300, p-value 0.000). In a different model specification, when including a family support variable in the subjective norm latent construct, the statistical significance and even the effect becomes borderline positive but with no statistical significance. Taking into account the importance of family support for Macedonian farmers, it is advisable to have that aspect within the subjective norm construct. This finding signals for deeper investigation of this issue.

Discussion and conclusions

The success of RD policy strongly depends upon the intention and ultimately the actual behavior of farmers in using RD funds. Following the TPB, intention is a function of farmers' beliefs related to RD program. Farmers in the survey with more positive attitude and more perceptive behavioral control have stronger behavioral intention to use the RD support available.

The positive attitude toward RD support strongly pronounced by farmers in Macedonia can be used when building RD policy strategies to increase the absorption of available support. The farmers mostly recognize the benefits affecting their individual farm household (e.g., income increase, farm survival). However, RD policy measures need to

also focus on common benefits that would improve the overall quality of life in rural areas and hence offer opportunities to farmers to participate in such joint projects.

Farmers perceived control over the process and self-confidence increases their intention to apply and is statistically significant. The better the abilities of the farmers, their control over information, knowledge and experience to independently prepare the RD support application, the higher the intention to request RD support. The procedure and rules for accessing RD support need to be simplified, more accessible and applicable with intensified extension support. Appropriately defined guidance and trainings can contribute to boosting farmer's self-confidence. Easier access to credit would additionally advance the use of RD support since the ability and preparedness to co-finance investments is an important factor.

Although the support from family is particularly important, this construct is not completely explained by the model. Further investigation of this issue and improved approach when eliciting beliefs from the target population can contribute to better understand and predicts this TPB component. The literature suggests that the subjective norm construct is generally a weaker predictor of intentions and behavior. Armitage and Conner (2001) argue that this occurs partly due to poor measurement and methodological approach as well as the need for expansion of the normative component. In agricultural related studies, the phenomena of social norm can be to some extent explained by the importance of independence to farmers (Gasson, 1973), making it understandable that farmers would not be very willing to declare in a survey that they are under social influence by the respected others (Burton, 2004). TPB proved to be an adequate tool for supporting policy analysis. The structural equation model measuring the influence of behavioral factors on intention and their interrelations gives an additional value. The application of these findings, as well as the methodological approach, in policy design and intervention can contribute to the effective use of the rural development funds.

Acknowledgements

The research is done within the regional project "The impact of socio-economic structure of rural population on success of rural development policy", prepared in the framework of the Regional Research Promotion Programme in the Western Balkans (RRPP), which is run by the University of Fribourg upon a mandate of the Swiss Agency for Development and Cooperation, SDC, Federal Department of Foreign Affairs. The views expressed are those of the authors and do not necessarily represent options of the SDC and the University of Fribourg.

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REASONS FOR INCREASED CONSUMPTION OF SLIMS CIGARETTESArsov Z.^{1*}, Kabranova R.¹, Kitanovski M.²¹University Ss. Cyril and Methodius-Skopje, Faculty of Agricultural Sciences and Food²International Tobacco Tasting Association-ITTA

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Abstract

According to the classification of individual consumption by purpose, from the State Statistical Office of Macedonia, alcoholic drinks and tobacco (such as tobacco products, cigarettes) in 2012 participated with 3.5% in the structure of individual consumption of households, while in 2014 notes their reduction to 3%. Dominant typical tobacco product for enjoyment here in Macedonia, traditionally is cigarette. Unlike earlier in the last years on the market appear cigarettes which have a different design (form-dimension). It so-called slims cigarettes, which are increasingly present on the world markets. The increase in supply with the participation of cigarettes with different formats like *Slims*, *Super Slims* and *Compact Super Slims* for tobacco companies has become a model for reducing the fall in the consumption of conventional cigarettes. Growth in Slims cigarettes consumption shows an upward trend on markets in several European countries. This upward trend is observed in R. Macedonia where starting from 2012 slims cigarettes consumption in terms of king size cigarettes from 1.86% in 2012 increased to 4.52% in 2014. As some reasons of increased consumption are: the design of cigarette, choice of brands and reduced smoking time. The appearance of other new products from the tobacco industry arouses interest, and research should evoke a new situation in the cigarette market. The aim of this study was to present the way in the creation and development of Slims cigarettes, and present relevant brands with their important functions, compared with other brands in the type of mix mixture. Also, we want to present the Macedonian national tobacco industry, especially in the consumption of tobacco products.

Key words: slims cigarettes, consumption.

Introduction

Cigarette consumption in the world over the last few years has stabilized at around 6.250 million cigarettes, or about 4.300 thousand tons of cigarettes. Also, the number of smokers worldwide is estimated at approximately 35% of the world's population or one billion for the same period (Figure 1), compared to predominant global trends for reducing the number of smokers. Chart 1, shows that in 1980 consumption of cigarettes in developed and developing countries was approximately equal to 2.59: 2.37 billion pieces. After 1990 cigarette consumption begins to increase in developing countries, and to decline in developed countries. Thus, in 2012 in developed countries cigarette consumption totaled 1.84 billion pieces, and in developing countries 4.41 billion pieces, i.e. increased by 2.4

times in terms of cigarette consumption in developed countries. Reasons for this phenomenon are mainly majority population growth and rising living standards in developing countries and increased antismoking campaign in developed countries. Compared to the world regions, largest consumer of tobacco is Far East with 47% of total consumption, followed by Europe with 21% and America with 11%. For the same period the number of smokers in developed countries has a downward trend, while in developing countries have seen an increasing trend, but the worldwide number remains unchanged. Around three in 10 men (31%) and one in 20 women (6%) now smoke daily compared with four in 10 men (41%) and one in 10 women (10%) in 1980. For the purpose, smoking and cigarette consumption to hold the same level, tobacco companies are forced in various ways to sustain the consumption of cigarettes.

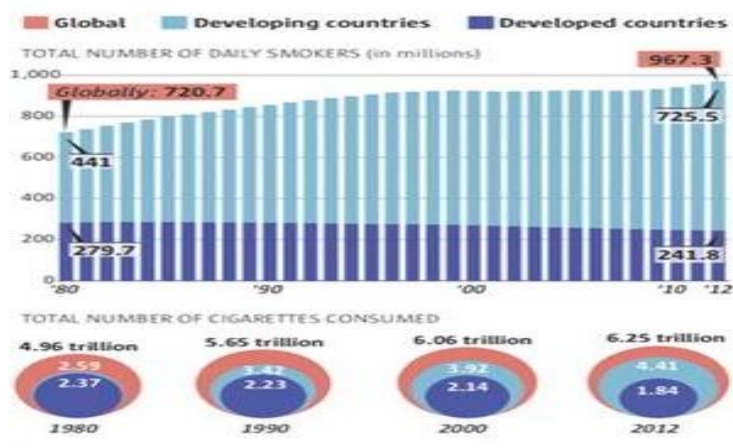


Figure 1.- Consumption of cigarettes and number of smokers in the world

Source *: 2012 SUPPLY & DEMAND Universal Leaf Tobacco Company, Inc. и The Seattle Times, January 7, 2014

Increasing the range of products with attractive cigarette characterized appealing design and a variety of formats-size (Moodie & Hastings, 2011) such as Slim, Super Slim and Compact Super Slim, has become a model to reduce the decline in consumption of conventional cigarettes size of 84 mm or 100 mm. Analyses of tobacco industry documents show that modifications to the appearance of cigarettes can make them more appealing to specific target groups, notably 'starter' and female smokers and can help to boost sales and market share (Cummings et al, 2002; Cook et al, 2003; Carpenter et al 2005). Exemplifying the potential for product design to influence growth, sales of slim cigarettes grew by 50% from 23855 million sticks in 2000 to 35673 million sticks in 2010 in Europe, despite a general decline in factory manufactured cigarette sales. This is consistent with global trends where the super slims segment is reported to have grown 10 times faster than the overall market in the past 5 years (European Commission, 2012).

Aim of the Study

The appearance of other new products from the tobacco industry arouses interest, and research should evoke a new situation in the cigarette market. The aim of this study was to present the way in the creation and development of Slims cigarettes, and present relevant

brands with their important functions, compared with other brands in the type of mix mixture. Also, we want to present the Macedonian national tobacco industry, especially in the consumption of tobacco products. We also want to present relevant information and data on consumption of ordinary and slim cigarettes in our country and abroad as the most important subject of research. We also wanted to present the reasons that led to increased consumption of slim cigarettes. On the other hand, we also presents the extension of relevant brands of slim cigarettes on a market area, as well as content of slim cigarettes (physical and chemical properties in certain cigarette brands, type, dimensions of cigarettes etc.).

Method of Work

The main method in this research is the presentation of data through statistic data processing by calculating the percentage values, calculation of average values for long-term (9 years), and calculation of the index pool from 2006 to 2014.

Consumption of tobacco products-cigarettes in Macedonia

The most common tobacco processing enjoyment in Macedonia traditionally is cigarette. Other types of tobacco products meant for enjoyment (cigars, cigarillos, pipe tobacco, chewing, snorting and hookah) are not as popular and are consumed in small quantities. According to the classification of individual consumption by purpose from State Statistical Office, in 2014, alcoholic drinks and tobacco (such as tobacco products, mainly cigarettes) accounts for 3% in the structure of individual consumption of households in cash or the equivalent of 10641 denars. The consumption of tobacco cigarettes and household and per household member for the period 2009-2014 is presented in Table 1.

Table 1. The consumption of cigarettes and tobacco for a household and per household member, 2009-2014 year

Year	Per household, the number of boxes*	Per member of the household, the number of boxes*	Per household, in kg **	Per member of the household, in kg **
2009	221	58	2,94	0,77
2010	183	48	2,43	0,64
2011	164	44	2,18	0,59
2012	154	46	2,05	0,61
2013	120	49	1,60	0,65
2014	132	36	1,76	0,48
Average	162,33	46,83	2,16	0,62

* Quantities of goods for personal consumption consumed in the household in annual average per household and quantities of goods for personal consumption consumed in the household, annual average per household member published in *Statistical Review: Incomes, Expenditures and Prices* of State Statistical Office Republic of Macedonia (2009-2014).

** Our calculation

If cigarette consumption calculated in kilograms and provisionally take that the box with standard length of 84 mm cigarette, on the market in Macedonia contains 19 cigarettes and weighs 13.3 g, or 0.0133 kg, in that case consumption by one family in kilograms was

greatest in 2009 when it was 2.94 kg, and the lowest in 2013 when it was 1.60 kg, i.e. we have a trend of reducing consumption and consumption in the last 6 years. Different data is obtained when we analyze the data for the production of cigarettes in both domestic cigarette factories, export and import of cigarettes (Table 2).

Table 2. Consumption of cigarettes in the country, tons

Year	Domestic production, tons	Export, tons	Import, tons	Domestic consumption, tons
2007	4114	1915	515	2714
2008	4439	2547	627	2519
2009	4480	2674	714	2520
2010	5064	3759	817	2122
2011	5711	4442	996	2265
2012	4861	3057	1268	3072
2013	4880	3032	1398	3246
Average	4792,7	3060,9	905,0	2636,9

Source: State Statistical Office of Macedonia

*Our calculation (production-exports + imports = consumption)

From the data presented in Table 2 may be noted that the consumption of cigarettes annually on the state level for the analyzed period is relatively stable and on average for the analyzed period amounted to 2637 tons, with fluctuations between 2519 and 3246 tons. It is noted that there is an increasing trend in 2012 and 2013. This increase in our opinion due to the impact on consumers from neighboring countries for various reasons comes to Macedonia and among other consumer products and buy cigarettes. During this period, prices of cigarettes in neighboring countries (Greece, Bulgaria and others.) are higher than the prices of the same brands of cigarettes sold in Macedonia. If it adds the illegal sale of cigarettes and tobacco for hand-folding, which the World Trade Organization (WTO) assesses that varies from 6-9% of the legal consumption worldwide if that rate is applied to the Republic of Macedonia (average 7.5% or 200 tons), we come to the figure of about 2900 tons annually. According to the number of male smokers, Macedonia occupies ninth place in the world with 46.5% and sixth place for women smokers (26.7%), data for 2012 (Ng et al, 2014).

History of Slims cigarettes

Cigarette (from the French word "cigarette", meaning cigar + ette = small cigar) is a product consumed by smoking, which is produced from dried and finely cut tobacco leaves and reconstituted tobacco, often combined with other additives, then formed in the shape of a cylinder filled in the paper, usually less than 120 mm in length and less than 10 mm in diameter (Georgiev, 2002; Nikoilić, 2004). Slim's name derives from the first mark of this form of a cigarette which appeared on the market, it's *Virginia Slims* and which target group was women. Primarily, the purpose of smoking is to enjoy, regardless of gender and age. But even as women consumers of tobacco products, they are more frequently and their differentiation regarding the use of tobacco products. In the past, wanting to be different from men smokers, female smokers mostly used long cigarette holder (long pipes) and thus manifested its elegance, femininity and sexual attractiveness. In the past, the female population of smokers are more often opted for cigarettes with greater length (83-84 mm,

100 mm and 120 mm), that acted more elegant than standard king size cigarettes which length is 84 mm with diameter of 7.5-8 mm. Then, consume cigarettes with less physiological effect and softer with greater presence of aromas. In recent times more women smokers continue to consume cigarettes, with the above mentioned taste features. But such cigarettes are already smaller diameter, which usually ranges from 5.4 mm to 6.1 mm and with greater length, 100 mm even and 120 mm. For the first time, this format of cigarettes of the brands Benson & Hedges and Virginia Slims appeared on the US market in the late seventies of last century by several companies in the US market, and they were primarily intended for women consumers (Weinstein, 1970). Countries in the Balkans, Slims cigarettes for the first time, massive emerging market in the period 1995-1996 year. At first it was mainly names of famous companies worldwide. Until today their participation in sales is growing. The number of different brands that appears in this format increases every year as it comes to an enviable offer from almost 20 -30% of the total supply of stamps for cigarettes to be positioned in offering Slims Super Slims and brands. In this market segment, the biggest contribution has multinational cigarettes companies. Significant share have some regional companies such as Karelia Tobacco Company (Moodie M. 2005), but also and others.

Brands of Slims cigarettes

Production and marketing of this form of cigarettes have multinational companies with their brands: More, Vogue, Capri, Virginia Slim and other products and they become recognizable products for women smokers. As in most developing countries and countries of the Balkan Peninsula trend of smoking Slims cigarettes is growing. In various European and world markets, also on the Balkan Peninsula, Slims cigarettes present the following brands: Davidoff Slims, Ronhil Slims, Karelia Slims, More, Eva Slims, Best Slims, Prestige Slims, Victory Slims, Assos Slims, Diwa Slims Rode Slims etc.

Usually on the market are found Slims cigarettes with characteristics presented in Table 3.

SECTION 2. AGRICULTURAL ECONOMICS

Table 3. The most common physical and chemical characteristics of slims cigarettes

Characteristics	Measure	King Size	Slims	Super Slims
1. Type of the blend--mixture		American or Virginian blend	American or Virginian blend	American or Virginian blend
2. Number of cigarettes in a box	number	19-20	19-20	19-20
3. Type of box		Soft; Hard box	Hard box	Hard box
4. Physical characteristics of the cigarette				
- Total length	mm	84-85	97	83
- Diameter of cigarette	mm	7,5-8	6,1	5,4
- Mass of a cigarette	g	0,70-0,80 +/- 10	0,72 +/- 10	0,62 +/- 10
- Mass of tobacco part	g	0,56-0,66 +/- 10	0,60 +/- 10	0,50 +/- 10
- Width of tobacco fibers	mm	0,6 - 0,8	0,4 - 0,6	0,4 - 0,6
5. Chemical features of tobacco smoke				
5.1. Nicotine	mg/cig	0,4; 0,6; 0,8-1	0,4; 0,6; 0,8	0,4; 0,6; 0,8
5.2. Tar in smoke	mg/cig	4; 6; 8; 10	4; 6; 8	4; 6; 8
5.3. Carbon monoxide	mg/cig	4; 6; 8; 10	4; 6; 8	4; 6; 8

Consumption of slim cigarettes

The production and consumption of Slims cigarettes in the countries of Central and Southeastern Europe in a relatively short period of time increased. In Table 4 which was accepted from the prestigious market researcher "Euromonitor", clearly shows the upward trend of growth of this type of cigarettes on that markets.

Table 4. Consumption of slim cigarettes

Country	Measure	2006	2007	2008	2009	2010	2011	2012	2013	2014	Average 2006-2014
Belarus	Slim/Superslim	250.70	405.40	724.5	1447.90	1527.50	1575.00	1615.90	1664.40	1725.00	1215.14
Belarus	Regular	16919.30	18539.60	19975.50	20162.10	20772.50	20925.00	20984.10	21135.60	21275.00	20076.52
Belarus	Total	17170.00	18945.00	20700.00	21610.00	22300.00	22500.00	22600.00	22800.00	23000.00	21291.67
BIH	Slim/Superslim	337.10	372.10	414.90	438.3	455.7	495.6	516.3	532.2	541.7	455.99
BIH	Regular	6466.10	6699.70	6890.40	6930.40	6894.70	7013.20	6965.70	6858.90	6879.30	6844.27
BIH	Total	6803.20	7071.80	7305.30	7368.70	7350.40	7508.80	7481.90	7391.00	7421.00	7300.23
Croatia	Slim/Superslim	63.50	101.2	101.4	76.2	108.1	139.8	153.6	207.1	219.3	130.02
Croatia	Regular	7942.30	8373.70	8417.20	8388.00	8206.10	8083.80	7931.50	7756.70	7612.90	8079.13
Croatia	Total	8005.80	8474.80	8518.60	8464.20	8314.10	8223.60	8085.20	7963.80	7832.20	8209.14
Czech	Slim/Superslim	481.30	1031.40	1378.10	1589.80	1786.90	1951.20	2065.10	2193.40	2202.80	1631.11
Czech	Regular	22435.90	22410.30	21589.80	22139.10	22360.10	22438.40	22519.30	23017.70	22547.40	22384.22
Czech	Total	22917.10	23441.80	22967.90	23728.90	24147.00	24389.60	24584.40	25211.10	24750.10	24015.32
Russia	Slim/Superslim	25868.70	39162.00	47220.00	49690.90	50361.70	52560.20	54972.20	57483.70	59506.20	48536.18
Russia	Regular	354553.40	333809.10	346280.00	332546.50	322688.00	322869.60	324146.30	325740.90	329423.00	332450.76
Russia	Total	380422.10	372971.00	393500.00	382237.40	373049.70	375429.80	379118.50	383224.60	388929.20	380986.92
Slovakia	Slim/Superslim	94.80	98.2	106.4	146.6	170	193.7	233	311	357.3	190.11
Slovakia	Regular	7195.20	7453.80	7491.60	7569.30	7558.20	7552.60	7533.00	7464.40	7410.10	7469.80
Slovakia	Total	7290.00	7552.00	7598.00	7715.90	7728.20	7746.20	7766.00	7775.50	7767.40	7659.91
Slovenia	Slim/Superslim	47.60	56.7	70.5	79.3	83.1	86.7	94.6	97.7	105.4	80.18
Slovenia	Regular	4712.70	4668.90	4630.60	4583.50	4535.40	4478.10	4411.10	4344.80	4284.40	4516.61
Slovenia	Total	4760.30	4725.70	4701.10	4662.70	4618.50	4564.80	4505.70	4442.60	4389.80	4596.80
Ukraine	Slim/Superslim	9754.20	9635.70	9333.50	10857.60	11083.50	11639.10	11791.20	12247.70	12777.70	11013.36
Ukraine	Regular	90804.60	87694.80	83815.40	77129.20	74835.00	73504.50	72794.30	71986.90	71120.40	78187.23
Ukraine	Total	100558.80	97330.50	93148.90	87986.80	85918.50	85143.60	84585.50	84234.70	83898.10	89200.60

If we calculate the percentage of thin and super slim cigarettes in the markets of the analyzed countries, based on data from Table 4, we provide data showing growth in the share of thin and super slim cigarettes from 2006 to 2014 (Table 5).

Table 5. Participation of slim and super slim cigarettes in total consumption of cigarettes

Country	2006	2007	2008	2009	2010	2011	2012	2013	2014	Average 2006-2014
Belarus	1.5	2.1	3.5	6.7	6.8	7	7.2	7.3	7.5	5.51
BIH	5	5.3	5.7	5.9	6.2	6.6	6.9	7.2	7.3	6.23
Croatia	0.8	1.2	1.2	0.9	1.3	1.7	1.9	2.6	2.8	1.60
Czech	2.1	4.4	6	6.7	7.4	8	8.4	8.7	8.9	6.73
Russia	6.8	10.5	12	13	13.5	14	14.5	15	15.3	12.73
Slovakia	1.3	1.3	1.4	1.9	2.2	2.5	3	4	4.6	2.47
Slovenia	1.00	1.2	1.5	1.7	1.8	1.9	2.1	2.2	2.4	1.76
Ukraine	9.7	9.9	10	12.3	12.9	13.7	13.9	14.5	15.2	12.46

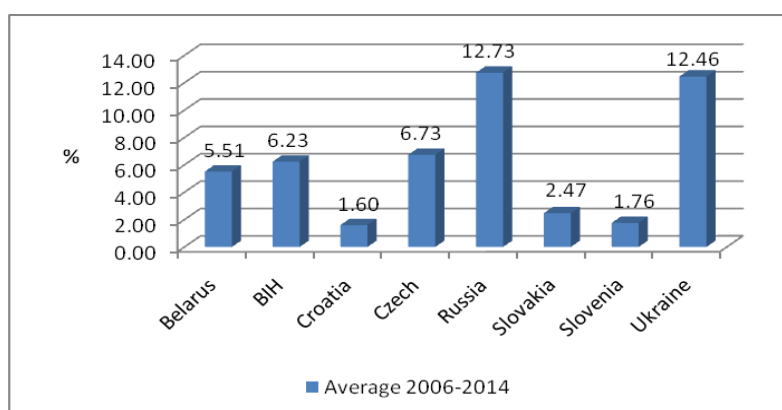


Figure 2. Share of Slims cigarettes in total cigarette sales in eastern and central European countries, average from 2006 to 2014 year

From the data presented in Chart 2, may be noted that for the analyzed period (2006-2014 year) Slim cigarettes have the biggest market share in Russia 12.73%, behind it is Ukraine with 12.46% while the smallest share in Croatia 1.60%. Data about consumption of slim and super slim cigarettes in Macedonia do not exist, the official statistics does not register. To determine, quantities consumption of slim and super slim cigarettes, we conducted a survey, which were obtained data on their consumption in the region of Tetovo. Indeed, it is one of the representative areas where spending cigarettes of different brands such as domestic and foreign, as well as a stronger cigarette price category. We surveyed four distributors of cigarettes wholesalers who are regularly present in the last few years and fully supplying the mentioned area (Figure 3).

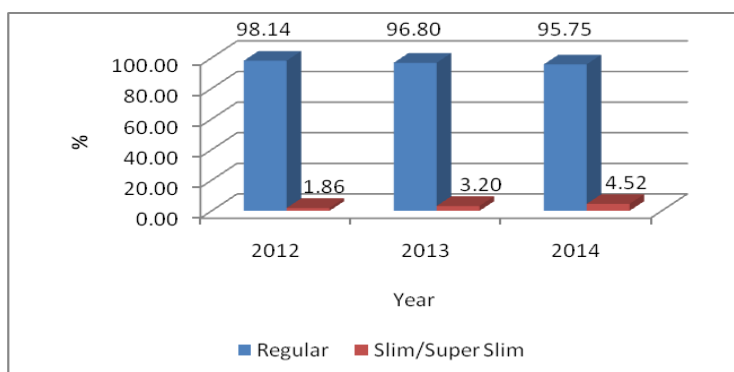


Figure 3.-Slim cigarette consumption in the Tetovo region, 2012-2014 year

From the data presented in Figure 3, may be noted that as of 2012 there was growth in consumption of Slims cigarettes in terms of king size cigarettes from 1.86% in 2012 to 4.52% in 2014, i.e. it is an increase of 2.4 times compared to 2012 year.

Reasons for increased consumption of Slims cigarettes

For rise of the production and consumption of Slims Super Slims cigarettes there are several important reasons.

- *Design as a reason*

The main reason for increased consumption is certainly the wish of modern (young) women to emphasize their femininity and sexiness using a more attractive appearance, "slims" cigarettes. They and their appearance and content differ from "male" standard king size cigarette diameter 7.8 mm and a standard length of 84 mm. As mentioned above, in general, these cigarettes come with relatively low physiological effect and almost never nicotine content does not exceed the limit of 0.8 mg / cig. Bestselling are Slims cigarettes with content of 0.4 mg to 0.6 mg / cig. Thus, the elegance of a cigarette almost after rule is accompanied by low content of nicotine, tar and carbon monoxide in the main stream of tobacco smoke and as such attracts the attention of female smokers.

- *Less harmful as the second reason*

A second reason for the rising trend of "slim" cigarettes could take the understanding of a particular group of smokers (male and female) that the belief that smoking such cigarettes reduce, mitigate the damage caused by smoking. They believe that cigarettes with diameter 6 mm or 5.4 mm have a lower content of tobacco raw materials and thus their harmfulness when smoking is significantly lower. This view is essentially correct, because the contents of burning is correlated with the mass of burned material. This is especially due to the above mentioned that in most of the samples, Slims cigarettes are low in nicotine, tar and carbon monoxide. So among smokers regardless of gender prevalent belief that these "slim" cigarettes are less harmful to health than conventional king size cigarettes.

- *Many brands from "smaller tobacco companies"*

The reason also for increased production and sales of Slims and Super Slims cigarettes will mention the fact that in a period of time in the late 90s of the last century the share of the dominant players in the tobacco industry (Philip Morris, BAT, JTI and Imperial Tobacco) was strong and undeniable. Smaller tobacco manufacturers their chance to take part of the market pie, saw in the introduction of brands, exactly this format (Slims and Super Slims) with that, in some way, they could compete with the 4 giants who in their arrogance felt

confident in its dominant position, they forgot that they should introduce innovations in production and supply. To illustrate and confirm this statement will quote the example of Karelia Slims brand, a product which appears in 1995 in the markets of Southeast Europe, as one of the few Slims cigarettes that were primarily intended for women (Moodie, 2005). This brand in a very short period of time popular on the markets of Russia, Ukraine and many countries of the Balkan Peninsula. Similar is the case of cigarettes Diana its share of the Italian cigarette market or ECCE which first appeared in South Korea after a relatively short time to become a leader in several countries in the Middle East.

- Shorter time of smoking as a cause of more frequent use of Slims cigarettes

One of the reasons for the increase in the use of cigarettes with small size is that the smoking time required for a present smoker is much shortened. Previously, smokers could smoke cigarettes in their workplaces and other public spaces unhampered by anyone. Now situation is different, smoking is forbidden in most public spaces, including working premises. Smoker to smoke his cigarette, now has little time and a little space. Therefore, the development centers of multinational tobacco companies have worked to follow the trend of "fast" smoking as a result of those efforts lately appeared cigarette with an extremely low content of tobacco. It is compact - Super Slims cigarette or briefly called Nano. Because BAT first launched this cigarette brand named KENT – Nano, then all cigarettes with this format briefly called Nano or Compact. This type of cigarettes, in this format, have tobacco mass of just about 0,35 g in a cigarette and blend in which is more content expanded tobacco and expanded tobacco rib, burning ends up after 6-7 withdrawals. So, the smoker for a short time (5 to 7 minutes) can return to his work room, a public place or restaurant. This type of cigarette appeared on the market in recent times, in the end of the first decade of the 21st century, i.e. they were introduced after the massive ban on smoking in the public places.

- Interest of tobacco companies for the development of this segment

On the other hand, this trend is also accepted and supported by the tobacco cigarette manufacturers themselves, primarily due to savings realized by the use of smaller quantities of tobacco. Reduction of tobacco at just over 0.10 grams per cigarette as production of 100 million pieces of cigarettes, would amount to 10,000 kg of tobacco. Also savings are achieved with intermediate goods used for production and packaging of cigarettes.

Conclusions

The consumption of tobacco in form of cigarettes for the period 2009-2014 calculated as average for a household is 162.33 boxes of cigarettes or 46.83 kg or 2.16 boxes of cigarettes or 0.62 kg per household member. For the same analyzed period there is a reduction in cigarette consumption. So, consumption of a household is 221 box (2.94 kg) in 2009, falling to 130 boxes (1.76 kg) in 2014, respectively, and decreases consumption per family member from 0.77 kg in 2009 to 0, 48 kg in 2014. Other data are obtained with analyze the production, export and import of cigarettes from Macedonia. According to these data there is no a downward trend in consumption of cigarettes. Cigarette consumption annually on the state level for the analyzed period 2007-2013 was relatively stable and on average for the analyzed period amounted to 2637 tons, with fluctuations between 2519 and 3246 tones. In the last few years in Macedonia slim cigarette

consumption is growing, like in the countries of Central and Southeastern Europe. According to our survey conducted in the region of Tetovo, the participation of slim cigarettes in total sales as of 2012 growing from 1.86% to 4.52% in 2014, i.e. it is increased by 2.4 times compared to 2012, and accordingly reduced sales King size cigarettes. In order to maintain cigarette consumption on the same level, tobacco companies are forced to keep consumption in various ways. Increasing the product range with attractive cigarettes that are featured attractive design and different-size formats such as Slim, Super Slim and Compact Super Slim model has become proven to reduce the decline in consumption of conventional cigarettes with a format of 84 mm or 100 mm. Women are the main target group of this format of cigarettes. Therefore, this format cigarette is elegant and with respect to smoking characteristics the main features are lower physiological force, greater presence of aromas and reduced smoking time compared to standard King size cigarettes.

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TOMATO PRODUCTION CHARACTERISTICS – COMPERATIVE ANALIYSIS IN EUROPEAN UNION COUNTRIES AND SERBIA

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Abstract

This paper analyzed tomato productions in some important producer EU countries. The analysis included six countries: Bulgaria, Greece, Spain, Italy, Portugal and Romania. The following production characteristics are analyzed and statistically processed: area of production, yields and total year production. Nominated countries encompass about 89% of total production of tomato in EU.

This research included official statistical data from „EUROSTAT“, for the period of 13 years, 2000-12. Descriptive statistical analysis included accountings of: average and extremes values of characteristics, coefficient of variations, and change rate. The results of analysis have been compared with the same data in Serbia. It is present tendency of decreasing the areas of tomato in all observed countries, except Portugal. The largest areas of tomato have Italy (122,000 ha), and Spain (60,000 ha). The average tomato area in Serbia in observed period was 21,000 ha. Opposite of areas, yields of tomato has tendency of increasing. The highest yields have Portugal (78.5 t/ha), Spain (69.5 t/ha), and Greece (54.5 t/ha). Average yield of tomato in Serbia is 8.6 t/ha. The all of observed EU countries have tendencies of decreasing production (except Spain and Portugal). The largest producer of tomato in EU is Italy, with about 6.4 million tons per year, in average. Next is Spain with 4.1 million tons.

Key words: tomato, production analysis, EU, Serbia

Introduction

Vegetables production is one of the most intensive branches of plant production, what proves high yields and high economic results. Taking in account importance of that branch of agriculture, in economic sense, for agricultural producers, and for agriculture in whole, basic directions for the future development are optimal usage of production capacity, increasing volume of production, and changes of production structure.

In this work, tomato production scharacteristics, in some, important producers countries in EU are analysed. The analysis included six EU countries: Bulgaria, Greece, Spain, Italy, Portugal and Romanija. The next productions characteristics are analysed, and statisticaly processed: area of production, yields, and harvested production. Nominated countries consist about 90% of total production of tomato in EU.

There are many papers about descriptive statistical analysis of vegetables (Mutavdzic et al., 2010, 2011; Novkovic et al., (2008, 2011, 2012, 2012a, 2014).

Novkovic et al. (2008) analysed vegetables production in Serbia and Vojvodina region, in the period: 1981-2007. Results are that vegetables production area in Serbia is 300.000 hectares in average. From that area, about 80.000 hectares is in Vojvodina region. Vegetables production in Vojvodina region is about 5% in the production structure of plough land, what is far from Serbia, where is that share 8, 5%. Area under vegetables is very stable. Tendency of low increasing is present in Serbia, and low decreasing is present in Vojvodina.

Mutavdzic et.al (2010), using regression models observe influence of yield and total production of some important sorts of vegetables in actual year, on sowing area in the next year. In general, in vegetables production, yield and total production don't have influence on sowing area in next year, as it is case of crops and industrial plants. The most, relatively important influence of yield, on sowing area in next year show melon & water melon, than paper, tomato and pea. Mutavdzic et.al (2011) analyzed natural results in vegetables production in Serbia in period 2001-10 year. Results of analysis are compared with adequate for the period from previous decade, 1992-2000. For last ten years, vegetables production in Serbia increase in all observed sorts of vegetables. Growth was from 2% in the case of onion, though 26% of paper, to 56% in the case of pea. Lazic (2014) analyzed and predict some production parameters for the ten, the most important kind of vegetables in EU. The goal of this research is to analyze tomato production parameters in main producer countries in EU, and see comparative position of Serbia.

Materials and methods

In this research the quantitative methods are implemented. Observed period of analyzed data is 2000-12. The data source is official statistical data from „EUROSTAT“, for EU countries. The descriptive statistic analysis included: average and extremes values of characteristics, coefficient of variations, and change rate. Tomato productions characteristics which are processed are: harvested area, yields, and total production. The results of analysis of EU in total, and main EU countries, have been compared with the same data in Serbia. Data source for Serbia are official publication of the Institute of Statistics of Serbia.

Results and discussion

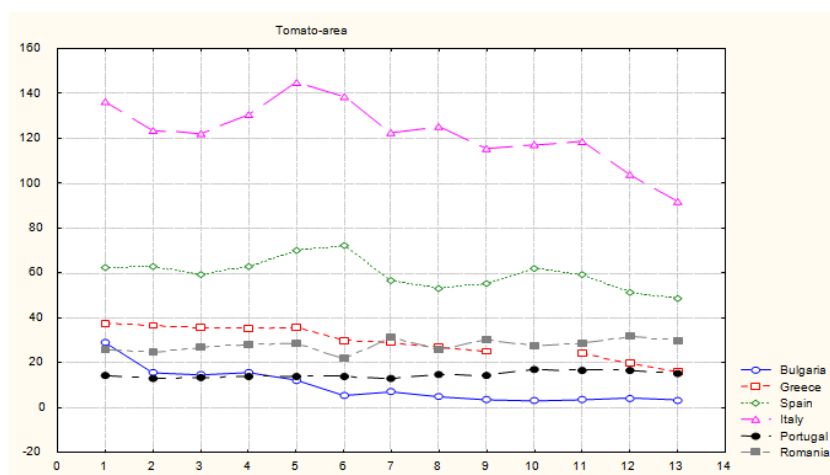
Average harvested area of tomato in EU in the period 2000-12 was 257,570 hectares. Area was changing from 229,600 to 293,100, from year to year. Coefficient of variation was very low, only 7.74%. Harvested area of tomato in EU in observed period shows tendency of slow decreasing, by the average rate of -1.38% per year. The main results of descriptive statistical analysis of harvested area of tomato in main producer countries in EU and Serbia are presented in table 1.

Table 1. Basic characteristics of tomato harvested area (2000-12)

Country	Average (000ha)	Interval of variation		Coefficient of variation (%)	Change rate (%)
		Minimum	Maximum		
Bulgaria	9.38	3.00	29.00	82.08	-16.36
Greece	29.24	16.00	37.40	24.19	-7.43
Spain	59.73	48.60	72.30	11.46	-2.05
Italy	122.38	91.90	145.00	11.59	-3.23
Portugal	14.62	13.00	16.80	9.31	0.62
Romania	27.75	21.90	31.60	9.97	1.21
Serbia	20.54	19.34	21.21	2.64	-0.67

Italy is dominant leading country in tomato production in Europe. Italy participate with, almost halve (47.5%) of harvested area of tomato in EU, in average, for the period 2000-12 year. Far, on the second place is Spain with 23% of total harvested area of tomato in EU. In tomato production is present tendency of decreasing of harvested areas in EU as total, and in all observed countries (except Romania and Portugal).

Serbia possess fifth place in harvested area of tomato, after Romania, and before Portugal. The very small coefficient of variation of harvested area of tomato is also positive, as a practically, stable tendency. The changes of harvested area in EU countries in the observed period are presented at graph 1. Average yield of tomato in EU in the period 2000-12 was 61,885 kg per hectare. Yield was changing from 58,430 to 65,460 kg/ha, from year to year. Coefficient of variation was even lower than in case of harvested area of tomato (4.84%). Yield of tomato in EU in observed period shows tendency of increasing, by the average rate of 2.5% per year.



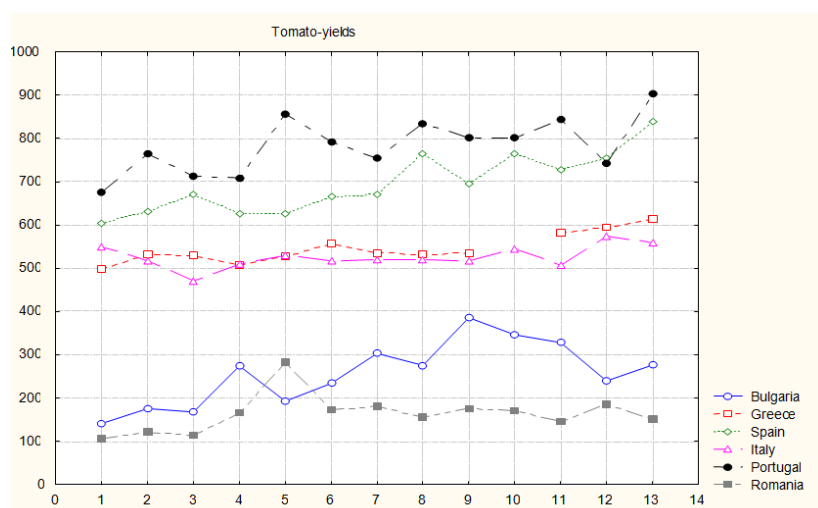
The results of descriptive analysis of yields of tomato are presented in table 2. Opposite of harvested area, yields of tomato in EU, and all leader EU producer countries and Serbia have tendency of increasing. Bulgaria, Romania and Spain have higher coefficient of change rate than average of EU change rate. The highest yields has Portugal, follow Spain, Greece and Italy. Bulgaria and Romania have a much lower yield of tomato. Only Portugal and Spain have a higher yield than EU average. Portugal for 27%, and Spain for 12%. Average yield

of tomato in Serbia is extremely low, far not only from maximal yields in EU, but also and far from average EU yield.

Table 2. Basic characteristics of tomato yields (2000-12)

Country	Average (kg/ha)	Interval of variation		Coefficient of variation (%)	Change rate (%)
		Minimum	Maximum		
Bulgaria	25,722	14,140	38,610	28.77	5.75
Greece	54,509	49,830	61,300	6.30	1.90
Spain	69,547	60,450	83,800	10.06	2.76
Italy	52,612	47,130	57,450	4.98	0.14
Portugal	78,456	67,650	90,410	8.40	2.45
Romania	16,410	10,640	28,220	26.83	3.03
Serbia	8,627	7,385	9,821	9.15	0.45

The changes of yield of tomato in EU countries in the observed period are presented at graph 2. Average year production of tomato in EU in the period 2000-12 was 15.716 million tons. Year production was changing from 15.029 to 16.091 million tons, from year to year. Coefficient of variation 3.01%, was the lowest, comparing with harvested area and yield of tomato. Year production of tomato in EU in observed period shows tendency of decreasing, by the average rate of -1.67% per year.

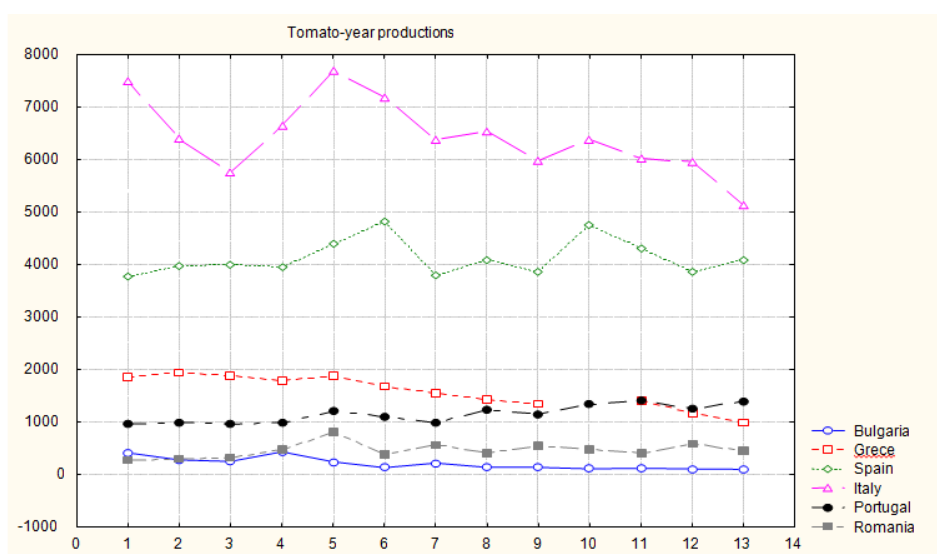


The results of descriptive analysis of total year productions of tomato are presented in table 3. Some of observed EU countries have positive tendencies of total production (Romania, Spain and Portugal), and some countries have tendencies of decreasing production (Bulgaria, Greece and Italy). The largest producer of tomato in EU is Italy, with almost 6.5 million tons per year, or more than 40% of EU production. Next is Spain with share of 26 % of EU tomato production. Average year production in Serbia is only 1.13% of total EU production of tomato.

Table 3 Basic characteristics of tomato year production (2000-12)

Country	Average (000t)	Interval of variation		Coefficient of variation (%)	Change rate (%)
		Minimum	Maksimum		
Bulgaria	201.26	94.00	428.20	56.72	-11.55
Greece	1,574.37	979.60	1,938.00	19.94	-5.68
Spain	4,122.70	3,766.30	4,810.30	8.34	0.66
Italy	6,424.90	5,132.00	7,683.10	11.06	-3.10
Portugal	1,149.94	954.70	1,406.00	14.55	0.03
Romania	459.25	274.60	805.10	31.47	4.26
Serbia	177.12	152.01	199.18	9.06	-0.23

The changes of year productions of tomato in EU countries in the obsrevd period are presented at graph 3.



Graph. 3 Tomato year productions (000t) (2000-12)

Conclusions

The results of tomato production analysis in main producers in EU, and Serbia show:

1. It is present tendency of decreasing the areas of tomato in EU and all observed countries (except Portugal and Romania). Total harvested area of tomato in EU is 257,570 hectares. The largest harvested areas of tomato have Italy (about 120,000 ha), and Spain (59,730 ha). Serbia with 20,540 hectares of tomato takes fifth place in Europe;
2. Oposite of harvested areas, yields of tomato have tendency of incereasing in EU in total, and in all leading EU counties, and Srebia. The average yield of tomato in EU was 61,885 kg per hectare. The highest yields had Portugal (78,456 kg/ha). Follow Spain (69,547 kg/ha), Greece (54,509 kg/ha) and Italy (52,612 kg/ha). Srebia, with yield of 8,627 kg per hectare is far from, even, average yield;

3. Tendencies of total production depends of changes in area of production and yields. EU has tendency of decreasing production of tomato. Observed EU countries show different tendencies. Tendency of increasing the production have Spain, Portugal and Romania, while Bulgaria, Greece and Italy have tendency of decreasing tomato production. Serbia also has symbolic negative change rate of tomato production. Average year production of tomato in EU was 15.7 million tons. The largest producer of tomato in EU is Italy (6.4 mill.tons per year), Spain (4.1 mil.t) and Greece (1.5 mil.t). Serbia with 0.2 million tons of year production of tomato is not significant producer in Europe, thanks to low yield.

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NETWORK STRUCTURES IN AGRIBUSINESS AS A NECESSITY TO INCREASE THE EFFICIENCY OF THE FARMERS

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Abstract

Interaction, collaboration, co-operation and integration within the agricultural sector are among the factors influencing the market structure and competitiveness of agriculture. One of the main reasons of the farmer for his participation in the network is to increase the efficiency of production, based on an effort to minimize production and transaction costs.

Key words: horizontal and vertical networks, clusters, contract farming, cooperatives.

Introduction

In a globalized economy, the Bulgarian producer faces simultaneously as serious challenges and competitive pressures on European and world markets, and to the chance to take advantage of new opportunities for their products and services. In this sense, Bulgarian entrepreneurs should focus on the implementation of profitable business strategies that make them competitive in the single European business space. Competitiveness in many more already dependent on the ability of firms to cooperation with potential partners from related industries, the complexity of corporate services, the efficiency of public administration and political solutions that help create a favorable business environment for economic development. The aim of the study is to present the characteristics of the sector and the need to create network structures and to clarify the benefits of participation in networks of farmer and rural areas.

The agricultural business includes a wide range of activities in the production and marketing of agricultural products. Depending on the purpose, these activities can be grouped as follows:

- proceedings, ensure the normal operation of the agricultural production process by providing him with equipment, fuel-lubricating material, fertilizers,
- activities carry on the agricultural production process;
- activities related to the storage, processing and marketing of agricultural products fresh and processed.

In the process of development of the agricultural sector, the importance of the areas which offer the supply of the business unit with the necessary raw materials (at the entrance), the realization of production, objective is increasing. By applying various forms of coordination, association, integration, cooperation and other increased the number and

importance of businesses closed cycle of production of the agricultural product in its processing to its ultimate realization.

Nature and basic types of networks

The main features and characteristics of agribusiness most often associated with the variety of activities and a long chain of entities between actual agricultural production and the end user (traders, processors, wholesalers and retailers). Together with the functioning of various sizes entities, the prevalence of a large number of small commodity producers and family nature of a significant portion of them are among the prerequisites for the development of specific agricultural policy for the public sector support.

Examined characteristics of agribusiness with the variety of combinations of natural and climatic factors, the large number of possible product combinations, and other markets are responsible for the operation of a wide variety of organizational units to farmers. They are created for different purposes, terms of existence, number of participants, operating mechanisms and management.

Among the most common are cooperatives contractual associations, producer organizations, clusters and others. They all create networks involving farmers to ensure sustainability of their farms.

The term network is termed relations of cooperation that arise between individual farmers or companies / organizations in the process of seeking compensation for the lack of resources and weak market position for sustainable development and provide a competitive advantage in the market, resulting in a synergistic effect. Network structure provides opportunities for the participation of organizations from all parts of the production chains in agribusiness. Thereby improving the implementation of processes and services from producer to end user and optimize the income of the participants in the network.

Network organizations differ mostly by the number of joint activities carried out and the type of existing connections. In some cases, network carried out a minimum number of activities (eg only transport and trade), and in others a number of interrelated and interdependent - picking, sorting, handling, cooling, transporting, processing, etc. In this relationship between the participants in the network can be relatively rare and uniform and continuous, dynamic and diverse. At the same time greatest importance and the degree of independence of each participant and mechanisms for deciding on the network.

Cooperation among network partners through the exchange of information, knowledge, profits and the risks and benefits offering opportunities for new contacts and markets, and expanding access to new or complementary skills and technologies (Lazzarini et al., 2001). Thus, SMEs are more innovative when they are able to join the network and participate in various activities. Successful cooperation is based on sharing information with all partners in the network, cooperative behavior among members of the network, and clear communication with well-defined goals and expectations. But also the sharing of risks and benefits in the network are the reasons for the successful cooperation, as it improves teamwork and focus on the common goals of all members of the network.

We can distinguish two different forms of networks. Horizontal networks involve cooperation of companies that are primarily competitors in the same sector. They cover initiatives such as strategic alliances or joint ventures to take advantage of opportunities for exchange of information, social services, based on informal connections (O'Donnell et al., 2001). To horizontal networks can be referred:

- Producer
- Cooperatives
- Contract farming

While vertical involve all stakeholders in the process of movement of agricultural products from producer to consumer. Vertical networks are formed with the participation of research organizations, government institutions and providers of financial services to all connected with a chain network. As a result, the network is rightly considered by some authors (Gellynck, Vermeire, Viaene 2006) as a place where internal and external resources of a company are combined and transformed into innovations. An example of such a network is a cluster.

Producer organizations

One option to overcome many of the problems farmers is the creation of producer organizations. Purpose of these structures is to stabilize production and improve the market position of producers. Pooling allows farmers to realize the benefits of unified production and supply.

According to some authors (Zaimova, 2008) farmers' organizations are producer groups, formed a territorial or product sign the initiative and voluntary choice of the producers themselves, who have a specific long-term goals and, regardless of the legal form their members work collectively their achievement. Other authors (Bijman and Wollni, 2008) offer the following definition: Producer organizations are formal organizations, membership is voluntary, created for the economic benefit of the farmers (members), providing services to producers who support their agricultural activities, provide technical assistance opportunities for processing and marketing services.

The inclusion of a producer organization and economic entities with in the manufacture and marketing of agricultural products is a prerequisite to use a number of advantages of vertical economic integration chain "land - finished product" (Kanchev et al., 2000).

Objectives should set a producer organization are mostly related to supporting the concentration of production and ensure its marketing. Furthermore, the producer organization must ensure its members that their production is planned and adjusted in accordance with market demand especially in terms of quality and quantity.

Technological development and environmental behavior of producers have other priorities of producer organizations. They encourage the implementation of new practices and production technologies as well as reasonable, environmentally sound waste management (eg grapes and wine) to protect the soil, water, landscape and biodiversity conservation. Thus the individual farmer is able to overcome problems relating to compliance with marketing standards for quality control and compliance, caused by small partidnost production and different quality through producers looking effects at sectoral and regional level. While sector they are mainly related to the adaptation of production to market demand, creating and balanced distribution of added value, competitiveness, etc., The regional, according to some authors (Zaimova, 2010) The advantages are achieved through improved grouping of supply; concentration of supply and improve the quality of production; guaranteeing producers' incomes; employment growth in the sector, investments in environmental protection and others.

Turn producer organizations have the right to unite in associations which are responsible for implementing common to all operational program and fund. Priority

activities of producer organizations should be to improve the production and marketing. This is especially important for small producers, which are difficult to manage production and market risk in the sector.

Cooperatives

Modern Cooperative theory considered cooperative broadly into three areas:

1. The cooperative as an association of people, the result of the limited capacity of the individual to survive in the external environment, and to satisfy their own needs.
2. The cooperative as a form of organization of social labor implies joint, simultaneous or sequential involvement in one or several interrelated labor or manufacturing processes.
3. The cooperative as an economic entity with its own immanent features to ensure its functioning in the business environment on the basis of its original management mechanism.

Each one of the three aspects of interpretation presupposes interaction and links between various participants in cooperatives. Some authors (Kanchev et al., 2000) defined as a democratic cooperative, voluntary association of people formed to achieve common economic, social objectives and other cultural goods. The originality of the cooperative as a business unit comes down to it, it is free association of individuals who have equal rights and self-governing. The cooperative is a need for family farms by pooling household in total business enterprise and stand for fundamental objective approving their production and commercial activities. In this respect, co-operation can be considered as a group of like-minded, producers and consumers, which are combined to allocate the results including and profit from their activities.

The uniqueness of the cooperative as a form of organization is associated with the system of principles which govern its operation.

Advantages of cooperatives:

- With their help manufacturers regulate part of its market weaknesses, both in terms of the role or offering agricultural products, and in terms of functions or traders;
- Cooperatives somewhat overcome the effects of the monopoly of a relatively small number of manufacturers of machinery, fertilizers, spare parts, etc.;
- cooperatives provide / perform much of the work processes of high technological level and the realization of its members economies of scale;
- cooperative provides various services to households from its members. Thus high quality services combined with their low price;
- Cooperatives are organizations that reduce economic risk and, as it is shared by all members in particular.

In practice commonly are 4 main criteria on which the classification is done on the cooperative organizational structures as: the attitude of the cooperative organization of the households of their cooperative members; of their business; the degree of closure of activity in independent economic enterprises and degree of integration or closing cycle of production processing.

Co-operation can be seen as a network of contracts. This approach considers the business relationship between the cooperative stockholders as contract relationships. It is a coordinating theory of agents economics of transaction costs and the theory of property rights / incomplete contracts.

Contract farming

The model of contract farming creates the conditions for access of farmers to production services and credit funds, as well as to knowledge and new technologies. Negotiating the price of agricultural products and the conditions of implementation are as a direct result of reducing risk and uncertainty.

Contract farming is defined as an agreement between farmers and processing and / or commercial companies. Broad opportunities that create a contractual form to reflect the specific situation and achieve the desired coordination between the two countries for the use of resources, relationship management, market behavior and others are reasons for its major distribution and importance in agribusiness. Contract farming helps farmers in the provision of market resources and specific management practices that will improve the quality of production. However, for businesses and individual entrepreneurs investing in the purchase of agricultural products in many cases is to participate effectively in such agreement than they organize own production. Thus, they provide the necessary amount of production quality that they themselves determine. Contract farming is seen as a way of reducing risk among farmers and processors or traders of agricultural production, as an opportunity to address market failures and reducing transaction costs, also provides opportunities to participate in markets for farmers who will not be available to them if they do not participate in the agreement.

According to some authors (Stoyanova, 2005) contract farming can be done on the basis of several models. The choice of each depends on the availability of markets for selling products, the availability of resources for the production and the requirements for a specific application and management of certain agricultural practices in crop cultivation or animal products.

Clusters

In the scientific literature, the most commonly used definition of a cluster of Michael Porter. It stresses that it is geographic connected network of similar interrelated or complementary competitive companies with active channels for business relationships, communication and dialogue, which share a specialized infrastructure, labor markets and services and are facing common opportunities development and / or threats. According Sefertsi (2008) participants in the cluster can be: as various business organizations and non-governmental organizations, educational organizations, scientific institutions, public administrations and individuals. Organized around a single unifying product or service and create a network that would be attractive to local and foreign investors. In their work Ron Martin and Peter Sunley (2003) drowsiness, submit a list of the ten most common definitions of clusters. Among these features that Roland and Hertag. According to them, clusters can be defined as networks of interconnected manufacturers without an element of spatial localization. Such an understanding reflects changing conditions of globalization and opportunities for creating international clusters.

Cluster rightly regarded by Michael Porter (2004) as a kind of self-organizing production system in which firms cooperate in vertical or horizontal "chains" to provide additional added value and increase competitiveness. It consists of a group of companies that usually have some experience of cooperation. However, most importantly, which can achieve the cluster is practical step towards business cooperation and incentives for the development of assets, technology, infrastructure and joint investments, effects that could

not be achieved by a single company. The creation of a cluster leads to significant cost savings for members of the network, the economic and social efficiency and others. Clusters are complex self-organizing systems and can be evaluated from different perspectives. Undoubtedly, the desire to find partners with whom to realize cooperation, coordination or separate activities along the value chain (technology development, production, distribution, marketing, service) and links between the participants should be institutionalized, constitutes a significant idea and its realization can essentially be seen as "organizational innovation".

In terms of membership of Bulgaria in EU cluster development is a key element of the national strategy for improving competitiveness. Exploring the relationship "competitiveness cluster", some authors (Kotzev, 2013) display five key competitive advantages. Along with the established conditions for the growth of labor productivity and reduce costs, create conditions for deeper specialization of labor and services of participating companies in the cluster for innovation and development cooperation.

The issue of clusters is becoming more urgent by the fact that small companies lack the capacity to meet European requirements and can not survive if we do not unite. Cluster ensure their markets and update technology and machinery. The experience of many countries shows that it is a way of survival for a small company that does not have the capacity to develop in different directions. As a result, the practice arose various forms of vertical integration.

The agricultural sector is one of the sectors with potential for development of clusters. The reasons are related, as the increased demand and prices for agricultural products, and the characteristics of actual production, processing and marketing of agricultural products. The benefits of the cluster approach for farmers in rural areas are as follows: increase in employment, training of employees, increase investment and innovation, lower costs of production cost and high added value. Successful cluster in the agrarian sector would gather many man farms with large producers in the area. Other participants could be processors of primary production, importers of agricultural machinery, various suppliers and manufacturers of chemical fertilizers and pesticides in agricultural holdings universities, transport companies and others.

Problems of the origin and functioning of network structures in Bulgaria

The reasons for the low interest for cooperation of small farms are diverse: lack of trust, lack of management skills marketing organization on their part (keeping of accounts management company / cooperative, etc.), The minimum required expenditure for the functioning the network and the like. It is therefore necessary to establish the legal basis to facilitate their cooperation by increasing their opportunities to occupy a strong position in the market for buyers; better opportunities for produced better prices, planning and reducing production costs, concentration of supply, a fairer distribution of value-added supply chain of food.

In order to promote the association of farmers, it is important to define terms and conditions for recognition of producer organizations, associations of producer organizations and inter-branch organizations in all sectors of agriculture, and the possibility of producer organizations to be created to achieve a variety of purposes. The application of such measures would lead to:

- significantly increase the number of recognized producer organizations;

- established organizations and producer groups to benefit from the economic advantages of association and increase their competitiveness in both domestic and external markets, as well as the possibilities for support under the Rural Development Programme (RDP) 2014 - 2020 г. ;
- increasing the role of organizations and groups of producers in the supply chain of food, including through a joint sale of products manufactured by their members, and increase the proportion of the added value that remains for farmers.

Analysis of the implementation of the Rural Development Programme (RDP) 2007-2013 under Measure 142 "Setting up of producer organizations" for the entire period shows that have been approved for support 4 producer organizations. The creation of producer organizations in agriculture and their development is one of the priorities of the Ministry of Agriculture and Food, assisted by the measure "Setting up of the producers' part of the Rural Development Programme (RDP). The measure aims to encourage the creation of producer organizations in the agricultural sector, to support the production and supply of high-quality products according to European standards and market requirements and to facilitate market access for small and medium-sized farms. Efforts to increase the degree of association of farmers do not work and the number of created in Bulgaria producer organizations is very small, and their market share is negligible.

Conclusions

Cooperation between farmers, processors and traders is weak due to lack of traditions, fragmentation of production, lack of appropriate forms and incentives for building long-term partnerships. This problem is an obstacle to long-term planning. Therefore, the promotion of cooperation between farms is a priority area of the RDP 2014-2020 to improve their market position, to increase their productivity through the optimal use of resources, and to be better able to take advantage of emerging market opportunities. Addressing this need requires the creation of groups and producer organizations and other forms of cooperation for joint marketing, storage and processing of production.

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PURCHASE OF AGRICULTURAL PRODUCTS AND THE IMPLEMENTATION OF THE PACKAGE UPIS

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Abstract

The subject of this paper is to show the importance of the purchase of agricultural products – cooperation and the possibility of its implementation in the information system enrollment for small and large businesses. Purchase of agricultural products, with its specifications, requires the possibility for flexible parameters defining and monitoring its implementation from contracting to paying. Based on the parameters defined in the current year, contracts with contractors and information on the purchase, follows the implementation of the contract and the quality of purchased agricultural products.

Key words: purchase of agricultural products, integrated information system, software package.

Introduction

Agriculture is one of the most important industries in Serbia. Agricultural production and food processing account for about 20% in the formation of GDP. Primary production is carried out mainly on small family farms, average size of 4.5 hectares. The share of these holdings in the structure of the purchase of agricultural products is higher than 50%.

Agricultural products market in Serbia is not so developed. This is reflected in the existence of monopolies, price instability, lack of organization of small producers, inefficiency of collection and distribution centers and the like. In order to improve this situation competent ministry has issued Regulations on minimum technical requirements for trade purchase places. Control of the organizations involved in the purchase performs inspection services of the ministry.

By the development of new software solutions in this field, as shown in the paper, the current situation can be significantly improved and enhanced. One of these solutions is a software package UPIS of the ERP class commonly referred to as "The First Serbian ERP." This software is the support of an integrated information system for planning and managing small and large business systems.

This paper shows a detailed description of the package relating to the purchase of agricultural products. It was explained that with its implementation many of purchase problems can be easily solved. The application is the parameter type, which allows further development and upgrading, depending on the needs of stakeholders involved in the purchase.

Specifications of purchase of agricultural products

The term purchase of agricultural products from the farm, means the purchase of agricultural products from family farms that carry out agricultural and other organizations for the purpose of selling, processing or refining. There are several types of purchases that are in some cases directly defined for a specific type of product:

- free purchase - purchase of agricultural products from independent production of family farms, based on the free supply and demand;
- collection services in agricultural products- agricultural and other organizations receive from family-owned farms as calculated value for the services of plowing, sowing, harvesting, threshing, grinding, replacement of wheat flour, etc., or delivered to the means of production;
- agreed purchase - taking over agricultural products from family farms under contract with the purchasing and other organizations taking and delivery of certain products (cereals, industrial crops, livestock, milk and other products);
- purchase of organized production - taking over agricultural products originating from the cooperation agreement regulates the production of family farms with agricultural and other organizations and taking over agricultural products of affiliated farmers.

The value of the purchase is paid in advance on the retrieved products. From that value of the purchase the costs of taking, transportation to storage, preservation and storage of purchased products are excluded. The value of the purchase does not include VAT, and premium that family farm receives for delivered quantities of certain products.

The competent ministry has issued Regulations on minimum technical requirements for trade purchase places in terms of space, equipment, devices, and methods of sale. The provisions of this Regulation do not apply to commodity exchanges, trade fairs, exhibitions and other sorts of traditional events, markets and wholesale markets [1].

Control the purchase and operation of organizations dealing with the purchase of agricultural products carried out by inspectors of the Ministry of Agriculture, Trade, Forestry and Water Management.

Purchasers are required to be registered at the place of redemption, make notice of all the relevant circumstances of the purchase: name and business name, a period when the purchase is made, the operating time of the purchase, the type of product being purchased, notice of the purchase price with VAT by Category (classes) and the time of payment.

These data have to be registered no later than 15 days from the date of purchase in Ministry of agriculture and trade. When purchasing buyer is obliged to issue a document that contains information about the purchaser and the farmer, the name, quantity and quality, or class of products purchased, the price of purchased products per unit of measure, date, time and means of payment.

Family farms have slightly higher participation in the implementation and purchase of agricultural products, than the companies and cooperatives (2009. - 51:49% , 2010. - 56:44% and 2011. - 57:43%)

Agricultural products market in Serbia is undeveloped, and this is reflected through the monopolization of demand, supply atomisation (a large number of relatively weakly associated small farmers), a large percentage of the market that is under unregulated flows of purchase and payment, price volatility of agricultural products, lack of purchasing and distribution centers and cooperatives, and inefficient system of stockpiles. The problem is that small companies cannot to organize the processing of the product and must agree with

the conditions that dictates the manufacturing industry [2]. In the last three years, milk production is for, these reasons decreased by 44 million liters

Law on Incentives in Agriculture and Rural Development and the Regulation on the allocation of subsidies in agriculture and rural development planned direct payments to producers in the 2013th year (maximum amounts):

- 1) premium for milk in the amount of 6.6 cents per liter of milk;
- 2) The main incentives for crop production are paid in the amount of 54 € per hectare;
- 3) incentives for quality breeding dairy cows in the amount of 180 € per head;
- 4) incentives for quality breeding sheep and goats in the amount of 36 € per head;
- 5) incentives for quality breeding sows in the amount of 36 € per head;
- 6) incentives for parents laying heavy type in the amount of 54 euro cents per head;
- 7) incentives for parents of small type chickens in the amount of 90 cents per head;
- 8) incentives for parents' party in the amount of 2.7 € per head;
- 9) incentives for quality breeding parent fish carp in the amount of 4.5 € per head;
- 10) incentives for quality breeding parent fish trout in the amount of 2.7 € per head;
- 11) incentives for cattle fattening in the amount of € 90 per head for fattening;
- 12) incentives for lambs in the amount of € 9 per head in fattening;
- 13) incentives for fattening pigs in the amount of € 9 per head in fattening;
- 14) subsidies for suckler cows in the amount of 180 € per head;
- 15) incentives for beehives in the amount of 4.5 € per hive;
- 16) incentives for the production of fish consumption in the amount of 6.6 cents per head;
- 17) the heating allowance in the amount of 54 € per hectare;
- 18) subsidy for the insurance premium for crops, fruits, perennial plants, nurseries and animals in the amount of 40% of paid insurance premiums;
- 19) reimbursement for the costs of storage in public warehouses in the amount of 40% of storage costs [1].

Serbia has very favorable natural conditions for diverse agricultural production (both crop, and livestock). The main agricultural products are corn, wheat, sunflowers, sugar beets, soybeans, potatoes, apples, plums, grapes, pork, beef and poultry meat and milk. Agriculture and food industry in the country's gross domestic product (GDP) accounted for about 17% (10.6% of agricultural production and the food industry 6.4%). However, if the overall contribution of agriculture to other sectors of the economy, especially the producers and processors of inputs and raw materials, is taken into consideration it can be seen that this share exceeds 40% of GDP. According to the Census of Agriculture (2012.), in the Republic of Serbia there is a total of 631 122 farms, including: 628 555 family farms and 2,567 (0.41%) holdings of legal entities and entrepreneurs. Farms used / processed 3,355,859 hectares of agricultural land and grow: 908,990 cattle, 3,403,288 pigs, 1,729,278 sheep, 235,576 goats, 673,651 and 26,627,308 poultry colony. Average farm in Serbia by 4.5 hectares of agricultural land, has a two-axle tractor, grown a head of cattle, four pigs, three sheep, 26 head of poultry and one colony.

SECTION 2. AGRICULTURAL ECONOMICS

Table 1. Production of field crops in Serbia - the 000 t

	Year				
	2008	2009	2010	2011	2012
wheat	2.095	2.068	1.630	2.076	1.921
corn	6.158	6.396	7.207	6.480	3.533
barley	344	303	244	279	266
sugar beet	2.299	2.798	3.325	2.822	2.328
sunflower	454	378	378	432	366
soy	351	349	541	441	281

Source: RZS [3]

Production of tobacco leaves in the 2012th amounted to 7,711 tons, of which the largest share cultivar Virginia with 84%, then 14% of Burley and Oriental 2%. This was significantly lower than in 2008. year when the production of tobacco leaves was 10839 tons. Produced 6,848 tons of fermented tobacco which is 6.5% more than in the 2011. Also produced 18,010 tons of cigarettes, which is 9.6% less than 2011th years, and this has affected the overall decline in the production of tobacco products by 6.7% compared to the previous year.

Vegetable farming is an important traditional economic activity in Serbia last year and there is still growing. In the production of the most common vegetables are potatoes, cabbage, melons and watermelons, tomatoes, peppers and onions.

Table 2. Vegetable production in the Republic of Serbia - t

	Year				
	2008	2009	2010	2011	2012
potato	844000	898282	887363	891513	577966
beans	42000	46337	43237	39508	22804
peas	42058	38895	36733	-	32697
tomato	176501	189353	189412	198677	-
pepper	151317	171366	154953	130104	-
cabbage	300519	326162	336600	315496	281557

Source: RZS [3]

Area under orchards cover about 240 285 hectares, which is 4.71% of total agricultural land and 5.69% of the arable land in Serbia. The largest percentage of the total number of trees is traditionally plum - 50%, apple - 18% and a cherry - 7%.

Table 3. Fruit production in the Republic of Serbia – 000 t

	Year				
	2008	2009	2010	2011	2012
apple	236	282	240	266	179
plum	607	663	427	582	391
cherry	90	105	66	91	75
raspberry	84	87	84	90	70
grapes	373	431	330	325	263

Source: RZS [3]

One of the peculiarities of Serbia is large share of sales in markets (29.8% of vegetables, fruits and grapes 20.3%, milk and dairy producer 17.6%, poultry and eggs 15.1%). In relation to the total value of the sale and purchase of agricultural products in

Serbian market, in the last year market share was 19.5%. It confirms that despite of growing competition, market kept its importance as an easily accessible, efficient and cheap distribution channel for agricultural producers. In regions Central and Western Serbian family farms at markets sells about 40% of its production in Southern and Eastern Serbia about 38%. At more than 400 markets in Serbia, with over 60,000 outlets, of which about 80% are stalls, about 80 thousand people are employed.

Structure of UPIS

UPIS - Management Business Information System is a set of programs ERP class for business application, from software house IIB from Belgrade. It is an integrated information system for planning and managing of small and large enterprises. This package is the result of years of working by experts of various profiles in order to include all segments of businesses of various profiles. The database is SQL server and the application of VisualFox-in. [1,2]

The basic properties of package are entry openness, inclusiveness, adaptability, connectivity related to security and reliability of the capabilities of process management, distributed work, work with other applications, multiple users work, continuous work, multilanguage support, operating in real time, graphical environment, multi-layered client server, data dictionary, detailed documentation. [1]

UPIS has useful applications, which function as a natural extension of the basic functionality of the main package in the areas of warehouse business, retail, web ordering and reporting, market research and field ordering, finally, a special application of advanced business intelligence (BI) [1]

Fig. 1. show the main application menu, with the basic subsystems that use a unique database

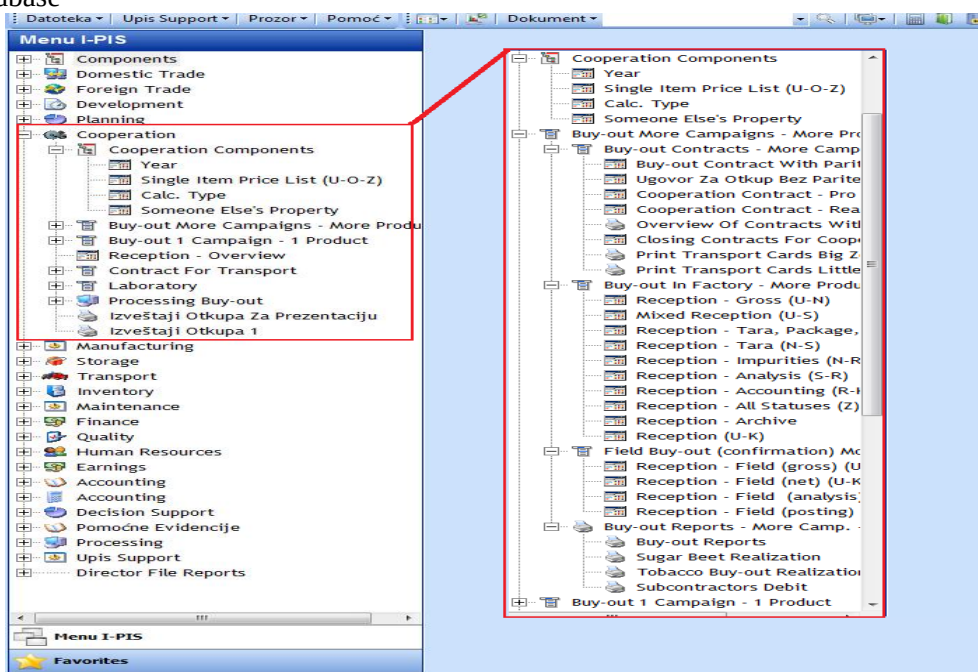


Fig.1. The main application UPIS menu

- Factors operations (Components) is a sub-system for keeping records of all the resources available in the system, ie. for all the codebook. His accuracy is a prerequisite for the functioning of other subsystems.
- Planning is a subsystem that covers the planning objectives of the work and funds (budgeting) and monitor their implementation. Depending on their purpose there are various operational and strategic plans. Based on available historical data, sale can be planed, from the sale production if the firm engaged in manufacturing, or supplying missing resources if the company deals only with trade.
- Development and design is a subsystem that covers the development, design and construction of products, tools and manufacturing processes. This subsystem is essential for all manufacturing companies.
- Marketing and Commercial in the domestic trade is a subsystem that monitors the demand, ordering, contracting, purchase and bill for all services, items and working objects, through the development of appropriate documentation and records.
- Cooperation in agriculture monitor contracting with or without financial obligation to the production of goods, delivery, receipt, purchase, consignment and exchange of agricultural products. Also monitor the results of laboratory analysis and history of use of agritechnical practices on the plots. This subsystem will be described in details in this paper.
- Foreign trade is a subsystem package of UPIS that allows monitoring of complete business, marketing and commercial at the international market, and the creation of appropriate documentation by the laws of the Republic of Serbia.
- Production (Manufacturing) is a subsystem that covers the operations of preparation, execution and calculation of production. Document preparation (work orders, requisitions, requests for procurement of raw materials, etc..) and the realization of production (worklist and transmitters of the finished product) can be launched on the basis of the production plan.
- Storage is a subsystem that covers the business of receiving and delivery of their own and other goods on discarded storage, packing, transport to the place of cost, delivery and distribution, as well as qualitative control of goods and its classification according to the results of quality control. Based on average daily sales and defined the minimum delivery time by a few suppliers, the usage of different test models is possible to determine the critical stock.
- Transport is a subsystem that runs operations of transport to customers and suppliers and launch the appropriate supporting documentation.
- Maintenance is a subsystem that covers the maintenance of the product during its use by customers, during the warranty and post-warranty period, as well as the maintenance of its own funds.
- Finance and accounting is a subsystem that performs business for providing funds and establishes the requirements for the company's liquidity and efficiency of operations.
- Personnel (Human Resources) is a subsystem that covers operations personnel records, from registering of candidates for the required jobs, their testing and employment, changes and career advancement, their departure from the company, to appropriate documentation.
- Earnings covers business payroll, records of all suspension, taxes, contributions, etc.. under the laws of the Republic of Serbia. The subsystem uses the common log of subsystem Personnel.

- Quality is a subsystem that covers the business of introducing and maintaining quality, editing documents, standardization of business, partner evaluation, quality control and monitoring of quality costs.

For each subsystem there are a number of reports, about 1,000 in the whole application. To facilitate the recording of business processes there are a number of automatic document formation that can form a document from the current document, the transition from the phase of processing to the final phase or stage records, or more documents based on completed documents according to certain criteria.

Given that enrollment connects all business segments and use heterogeneous enterprise it is constantly evolving and it is being updated with new functionality.

Subsystem cooperation in agriculture

Because of the specificity that characterize the production and delivery of primary agricultural production, special subsystem Cooperation was singled out [6]. UPIS has been used by companies that purchase: tobacco, sugar beets, sunflowers, corn, wheat, barley, corn, fruits (raspberries, blackberries, strawberries), milk. All these products have their own peculiarities, both in purchase and in laboratory analysis, and recalculated purchased weight, and all of them are provided with this package. This fact allows companies to purchase different products using a single information system.

The basic parameters of the purchase of the product at the crop year can be defined in (Cooperation Components). The first step in defining these parameters is to determine the method of calculation. Each calculation has its own code and name, the type of analysis of agricultural products, the order of measuring and recording mode, the input price when submitting the product and the model of calculation surrender amount. Model calculation of surrender amount usually depends on the product characteristics that influence the amount which is recognized to manufacturer for payment. Besides these data are defined by characteristics that accompany the purchase, as well as their values. On the Fig. 2 shows the form with two methods of calculation, milk and wheat.

The screenshot displays the UPIS.NET application interface with two overlapping windows. The top window, titled 'UPIS.NET - RACIOVI VELIKOJ URSIA 1109', is for defining parameters for 'MILK'. It shows fields for Code (015), Name (OTRUP MILKA), Agricultural product (MILK), Monitor the weight (27N22), Input price method (02), and Calculation model (003). Below these are two tables: 'Characteristic processing type' and 'Harvest Year - Class'. The bottom window, titled 'UPIS.NET - RACIOVI VELIKOJ URSIA 1109', is for defining parameters for 'WHEAT'. It shows fields for Code (005), Name (MILKA - LABORATORIA), Agricultural product (WHEAT), Monitor the weight (27N22), Input price method (02), and Calculation model (002). It also displays two tables: 'Characteristic processing type' and 'Harvest Year - Class'. The 'Harvest Year - Class' table in the bottom window has a red circle around the 'Price' column, highlighting the values 2.0000, 1.1200, and 0.0000.

Fig. 2. Types of accounts and crop year

When the model of calculation is defined, for each crop year it is possible to define a general product and specific products for which the purchase document entries. Fig. 2 shows the form for entering the data. This is especially important for products for which the surrender determines the class to which they belong.

At the level of general products entered into contracts, one can enter subcontractors with the planned amounts for surrender. Conclusion of the contract is not mandatory but it is useful to group together around all the documents in one year of purchase. In addition, for agricultural production where subcontractors provide advance materials (seeds, fertilizers, plant protection, etc.) or machinery for production, it is necessary to connect this deal with contracts that define how to pay received materials or machinery. The payments may be monetary or in goods, annual or perennial. For these contracts at the level of the crop year, price list of replacement of materials, and machinery with goods can be defined. If in the agreement are recorded parcels using a subcontractor, it is possible to monitor agricultural practices and crop rotation on the plots, regardless of whether they change owners every year. In addition to contracts with subcontractors, it is possible to record contracts with carriers, with transport prices which usually depend on mileage and cargo volumes. These contracts and related services for the transport can be recorded at the surrender of the product. Implementation of purchase is monitored through the required number of phases. In the case of delivery as usual are following steps: weighing the truck with the product, sampling for laboratory analysis, unloading and weighing exit the truck, the calculation amount purchased from laboratory analysis and weighing of transportation funds. In the case of shipping their order is different. The type of calculation defined weights which are recorded on a document of surrender, so called scaling list. Each transition from one phase to another corresponds to the menu item. For each customer application can defined stages that he can see, what he can do with the permit work (enter, modify, delete, looking).

Purchase document (weighing) occurs with truck coming from the field or from other buying center to the factory for processing of agricultural products. Each entry gets the number of weighing and all the necessary information is recorded to identify what was purchased, when was purchased (date and time), with any redemption of the carrier. The first weight, which is defined in the type of calculation that is assigned to the product data for crop year is recorded. Weighing data can be saved or downloaded, depending on the definition of redemption. Fig. 3 shows the form track delivery/ shipping of agricultural products.

The figure consists of two screenshots of a software application. The left screenshot shows a window titled 'Prilozak - Izdavanje DOK'. It contains several input fields for 'Prilozak', 'Datum', 'Godina', and 'Mesto'. Below these fields is a section titled 'Kontrola Prilozak' which includes a table with columns for 'Mesto', 'Godina', 'Bruto', and 'Neto'. The right screenshot shows a window titled 'Prilozak - Bruto UN'. It also contains input fields for 'Prilozak', 'Datum', 'Godina', and 'Mesto'. Below these fields is a section titled 'Kontrola Prilozak' which includes a table with columns for 'Mesto', 'Godina', 'Bruto', and 'Neto'. Both windows have a 'Kontrola Prilozak' section at the bottom with a table for recording data.

Fig. 3. Form for delivery of agricultural products-weighing

Conclusions

Given the importance of raw materials in the food industry the purchase is one of the most important segments of biotech and financial operations. Integrated information system that contains a module for the purchase is a necessary condition for the functioning of the primary production and processing. In addition, this subsystem is defined to connect to potential information integrated monitoring system of agricultural production in the whole country, which, unfortunately, does not exist in the Republic of Serbia yet.

Subsystem that is presented in this paper has been implemented in a number of processing companies for the purchase of primary agricultural products. It allows monitoring of all data on purchases set by the Ministry of Agriculture as well as all that is necessary for successful management of companies, regardless of the type of operations.

Acknowledgements

This paper was written within the project of technological development TR31051 financed by the Serbian Ministry for Education, Science and Technological Development.

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Nomenclature (Abbreviations)

RSZ Statistical Office of the Republic of Serbia

ERP Enterprise resource planning

RS Republic of Serbia

CONDITION AND PRODUCTION CHARACTERISTICS OF SHEEP AND GOAT BREEDING IN THE AREA AT THE CONFLUENCE OF THE RIVER BREGALNICA

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Abstract

The area of the river basin Bregalnica covers 14% of the territory of Macedonia, with an exact area of 3.537 km². The overall agricultural land is 162.480 ha (14.5% of the total agricultural land in the state), from which 84.840 ha (52.2%) is pastures. Calculated according to the average for sheep and goat breeding (2-5 heads / ha), the capacity of pastures in this area is 169.680 to 424.200 sheep and goats. The current number of 156.092 sheep and 18.904 goats in 2013 speaks of a number that is far below the existing capacity for raising these animals in this region.

This clearly demonstrates that this region has the potential for development of these two farm sectors. Significant reduction of the pastures surfaces is performed with the latest actions for forestation of many hilly areas in the country, including those at this region, making it even clearer that the resources for sheep and goats are being permanently reduced. The existing breeders are facing serious problems mainly because of the reduction of the grazing area.

According to the urbanization, the region includes 289 villages, from which 279 places are designated as rural settlements. The total number of residents is 206.380, and the population density is 58 inhabitants / km². There are lots of depopulated places, like villages with less than 100 inhabitants and villages with high index of aging. This situation led to a concentration of about 66% of the population in the urban areas.

Keywords: product characteristics, sheep breeding, goat breeding, pastures, basin area

Introduction

Sheep and goat breeding in the Republic of Macedonia are livestock industries with centuries-old tradition. Their development depends on the environmental and the social factors. Environmental factors in most of the regions in the country, including the region of the confluence of the river Bregalnica, created such a type of vegetation that is most suitable for sheep and goats, so the population of this region has also adapted and organized this type of economy. The social element is in second place, because sheep and goats for centuries have been providing basic subsistence of the population.

Production systems in sheep and goat breeding (extensive and intensive) are a result of the climate conditions, economic characteristics and planned size of the farms. In

the Republic of Macedonia dominates the stationed extensive system of sheep breeding (85-90%), with a small part of the nomadic system (10-15% of sheep breeding), although recently there have been attempts to intensify the sheep breeding in a few new farms. At some part of the farms, the sheep from the middle of May to early November are staying in the highland pastures, and during the rest of the year in the winter pastures.

Sheep and goats consume exclusively plant foods, mainly of grasslands, bushes, etc., so the food with low nutritional value and energy, converts into products with high value of protein: meat, milk, wool and leather. Because of that, we can say that sheep and goat breeding are the cheapest and most economical livestock branches.

Sheep breeding in the Republic of Macedonia is a traditional business, from which the main products are: dairy lambs and sheep milk (which is passed on in the dairies or it is processed into white soft goat cheese or sheep's yellow cheese). There's an interesting fact that the majority (60-70%) of the milk production is processed at home, and the rest is sold to the dairies.

Matherial and method

The basic data source in our study is the Statistical Office of the Republic of Macedonia through statistical yearbooks from specific years, statistic reviews of Agriculture, and also data received from the Agency for Veterinary and Food, which allowed us access to its records for labeled cattles in the municipalities and villages in Macedonia. We used many statistic reviews and statistical yearbooks for the past 23 years, i.e. regular censuses of population and property of the agricultural holdings, number of the livestock, etc.

Because of the comparability between the events in the development processes, we used numerous publications by well known authors, which were given in the list of references.

During the period of the study, we used mainly the inductive-deductive method, method of indexes, comparative method, averages, and more. For better perspective of the cattle number, the data is arranged in tables.

For easier understanding of the situation of the livestock in the region of the confluence of the river Bregalnica, we have shown the number of the livestock in the country and the one in the watershed parallelly. Our opinion is that in this way the perception will be clearer and more descriptive, so you will be able to see the current situation, and also to conclude where should be taken the necessary measures to fix this existing situation.

According to Table 1, there are 19 municipalities with 288 settlements in the watershed, and there are 206.380 inhabitants. While, according to the latest census in the Republic of Macedonia in 2002, 2.022.547 people live here.

Table 1. Municipalities in the confluence of the river Bregalnica

	Municipalities	Number of settlements
1	Berovo	8
2	Vinica	16
3	Gradsko	1
4	Delcevo	21
5	Zrnovci	3
6	Kriva Palanka	1
7	Karbinci	29
8	Konce	14
9	Kocani	28
10	Kratovo	8
11	Kumanovo	1
12	Lozovo	11
13	Makedonska Kamenica	9
14	Pehcevo	7
15	Probistip	37
16	Radovis	3
17	Sveti Nikole	34
18	Cesinovo - Oblesevo	14
19	Stip	44
	TOTAL	288

Source: Statistical Office of the Republic of Macedonia

According to Table 2, the total number of sheep in the country in 1991 was 2.250.549, while in 2013 that number dropped to 731.828. The decrease is 1.518.721 sheep or 67.48%. The total average number of sheep for a period of 23 years is 1.401.228.

The number of lambs up to 1 year in 1991 was 490.862, while in 2013 that number dropped to 148.441. The decrease was 342.421 lambs or 69.75%. The total average number of lambs up to 1 year for a period of 23 years is 294.030.

The number of sheep for breeding in 1991 was 1.623.242, but in 2013 dropped to 508.942. The reduction was 1.114.300 sheep or 68.64%. The total average number of sheep breeding for a period of 23 years is 1.008.102.

The number of rams and barren sheep in 1991 was 136.445, and in 2013 that number dropped to 49.482. This reduction is 86.963 heads, or 63.73%. The total average number of rams and barren sheep for a period of 23 years is 98.011.

Number of sheep in the area of the confluence of the river Bregalnica

The region of Bregalnica's river basin covers the catchment area of the river Bregalnica and covers 14% of the territory of the Republic of Macedonia, with a clear area of 3.537 km². The total agricultural land in this area is 162.480 ha, ie 14.5% of the total agricultural area in the country. Out of this, 84.840 ha or 52.2% are pastures. According to the average number for sheep and goats on a ha of pasture (2-5 heads/ha), the capacity of pastures in Bregalnica's river basin is 169.680 to 424.200 sheep and goats. The current figure of 156.092 sheep and 18.904 goats in 2013, speaks of a number which is far below the existing plant capacity of these species in the region.

This clearly demonstrates the potential that this region holds for the development of these two farm branches. Significant reduction of pasture surfaces is performed with the

latest actions to reforest large number of hilly areas in the country, including those at the confluence of the river Bregalnica, which once again confirms the fact that the resources for sheep and goats in the country are permanently reduced. Thereby, the existing breeders of sheep and goats are facing serious problems in breeding, primarily due to the reduction in grazing areas for various reasons.

As a potential for the development of these livestock industries, especially the goat breeding, are the forests (referring to already established forests) which at the confluence of the river Bregalnica cover 135.378 ha, or 13% of the total forests in the Republic of Macedonia, which is 38% of the total area in the region. In young forests breeding is forbidden (for all types of economic useful animals), because it can cause a lot of damage.

According to the urbanization, the region includes 289 populated areas, of which 279 places are designated as rural settlements. The total number of inhabitants in the region is 206.380 and the population density is 58 inhabitants / km². This region has almost four times lower population growth in the country. There is a huge number of displaced areas, ie villages with less than 100 residents and villages with particularly high index of aging. This situation led to a concentration of about 66% of the population in the urban areas.

According to Table 2, the total number of sheep in the region of Bregalnica's river basin in 1991 was 489.269, while in 2013 that number dropped to 156.092. The decrease is 333.177 sheep or 68.09%. The total average number of sheep for a period of 23 years is 304.668.

The number of lambs up to 1 year in 1991 was 97.853, while in 2013 that number dropped to 31.218. The reduction is 66.635 lambs or 68.09%. The total average number of lambs up to 1 year for a period of 23 years is 60.933.

The number of sheep for breeding in 1991 was 342.488 and in 2013 dropped to 109.264. The decrease is 233.184 sheep or 68.08%. The total average number of sheep for breeding for a period of 23 years is 213.267.

The number of rams and barren sheep in 1991 was 48.928, while in 2013 that number dropped to 15.610. This reduction is 33.318 sheep, or 68.09%. The total average number of rams and barren sheep for a period of 23 years is 30.468.

SECTION 2. AGRICULTURAL ECONOMICS

Table 2. Number of sheep at the confluence of the river Bregalnica

Year	Total number of sheep	Lambs up to 1 year	Sheep for breeding	Rams and barren sheep
1991	489.269	97.853	342.488	48.928
1992	504.847	100.969	353.392	50.486
1993	537.706	107.541	376.394	53.771
1994	544.514	108.902	381.159	54.453
1995	504.347	100.869	353.042	50.436
1996	389.443	77.888	272.610	38.945
1997	356.707	71.341	249.694	35.672
1998	290.390	58.078	203.273	29.039
1999	280.170	56.034	196.119	28.017
2000	268.522	53.704	187.965	26.853
2001	281.051	56.210	196.735	28.106
2002	268.234	53.646	187.736	26.852
2003	266.084	53.216	186.258	26.610
2004	311.397	62.279	217.977	31.141
2005	267.088	53.417	186.961	26.710
2006	271.489	54.297	190.042	27.150
2007	180.511	36.102	126.357	18.052
2008	178.591	35.718	125.013	17.860
2009	166.782	33.356	116.747	16.679
2010	169.300	33.860	118.510	16.930
2011	164.643	32.928	115.250	16.465
2012	160.200	32.040	112.140	16.020
2013	156.092	31.218	109.264	15.610
Average	304.668	60.933	213.267	30.468

Source: Statistical Overview: Agriculture, Animal Breeding 2007, 2008, 2009, 2010, 2011, 2012, 2013 p. 19, 20, 35 and data from the Agency for Food and Veterinary

Racial composition of sheep in the river basin of Bregalnica

According to Table 3, the dominant population in the region of Bregalnica is the Pramenka breed - Ovchepolian strain (53.962), followed by the sheep deployed in another population (34.000), than cross-breed Wurttemberg (29.583), Pramenka breed - Sharplaninian strain (19.000), Wurttemberg (18.510) and Cross-breed Avasi (9.443). The other types of breeds and cross-breeds are represented in a very small number that is almost neglecting in terms of so far mentioned types of races. Shortly, other races and populations are represented by insignificant numbers.

Table 3. Racial composition of sheep in the river basin of Bregalnica in 2010/2011

Breed	Number of sheep
Pramenka breed - Ovchepolian strain	53.962
Pramenka breed - Sharplaninian strain	19.000
Other population	34.000
Cross-breed Wurttemberg	29.583
Wurttemberg	18.510
Cross-breed Avasi	9.443
Avasi	45
Pramenka breed - Karakachan strain	50
Eastern - fresian breed	20
Cross-breed with eastern - fresian breed	20
Blackhead - pleven breed	10
Total	164.643

Source: Agency for Food and Veterinary

There are sheep farms all over the area at the confluence of the river Bregalnica, specifically in the following municipalities and villages: the villages Budinarci, Vladimirovo, Rusinovo and the city of Berovo in the municipality of Berovo, the villages Crnik, Negrevo, Robovo and the city of Pehcevo in the municipality of Pehcevo, the city of Vinica, the villages Bigla and Rizlovci in the municipality of Delcevo, the villages Gorni Balvan, Dolno Trogerci, Kucica, Prnalija and Radanje in the municipality of Karbinci, the villages Gabrevci and Dolni Lipovikj in the municipality of Konce, the villages Durfulija, Malino and Kjoselari in the municipality of Lozovo, the village Sasa in the municipality of Makedonska Kamenica, the villages Gorno Barbarevo, Lezovo, Strisovci and Troolo in the municipality of Probistip, the village Topolnica in the municipality of Radovis, the villages Amzabegovo, Delisinci, Erdzelija, Kadrifakovo, Meckuevci, Mustafino, Nemanjica, Orel and Sopot in the municipality of Sveti Nikole, the villages Banja, Vrbica, Sokolarci and Teranci in the municipality of Cesinovo-Oblesevo, the villages Baltalija, Dolani, Dragoevo, Krivi Dol, Leskovica, Sarcievo, Sofilari, Star Karaorman, Suvo Grlo, Sudikj, Susevo, Cardaklija and Sasavarlija in the municipality of Stip. In the summer, part of the sheep resettle in the villages which have mountain pastures, while in the autumn after November 15, they come down in the plain areas of the settlements. This way of cattle breeding is semi - nomadic and in the summer grazing they use the highland pastures in their municipalities.

Production of sheep milk at the confluence of the river Bregalnica

According to the data in Table 4, the total milk production in the basin of the river Bregalnica in 1991 is 43.843 million liters, while in 2013 that amount increased to 63.428 million liters. The increase is 19.586.000 liters or 44.67%. The total average milk production for a period of 23 years is 58.311 million liters of milk collectively.

The total cow milk production in 1991 was 31.171 million liters, while in 2013 that amount increased to 55.889 million liters. The increase is 24.718.000 liters or 79.29%. The overall average production of cow's milk for a period of 23 years is 47.648 million liters.

The overall milk production per milk cow in 1991 was 1.301 liters, while in 2013 that amount increased to 2.806 liters. The increase is 1.505 liters per cow or 115.68%. The total average milk production per cow for a period of 23 years is 2.116 liters.

Table 4. Production of cow and sheep milk at the confluence of the river Bregalnica

Year	Total milk production in 000 liters	Total production of cow milk in 000 liters	Liters per milk cow	Total production of sheep milk in 000 liters	Liters per milk sheep
1991	43.843	31.171	1.301	12.672	37
1992	42.525	30.157	1.300	12.368	35
1993	42.442	30.398	1.320	12.044	32
1994	46.930	33.590	1.278	13.340	35
1995	47.428	33.660	1.400	13.768	39
1996	44.577	33.673	1.401	10.904	40
1997	43.057	32.820	1.390	10.237	41
1998	49.657	41.527	1.890	8.130	40
1999	61.041	53.001	2.100	8.040	41
2000	58.140	49.870	2.305	8.270	44
2001	54.870	45.034	2.081	9.836	50
2002	54.629	43.741	2.056	10.888	58
2003	57.758	46.769	2.120	10.989	59
2004	64.848	54.822	2.303	10.026	46
2005	56.948	46.479	2.207	10.469	56
2006	64.912	53.320	2.460	11.592	61
2007	65.843	58.009	2.802	7.834	62
2008	66.304	58.304	2.800	8.000	64
2009	77.892	70.304	2.980	7.588	65
2010	66.112	59.357	2.690	6.755	57
2011	66.372	60.725	2.805	5.647	49
2012	67.578	59.392	2.877	8.186	73
2013	63.428	55.889	2.806	7.539	69
Average	58.311	47.648	2.116	10.663	50

Source: Statistical Overview: Agriculture, Animal Breeding 2007, 2008, 2009, 2010, 2011, 2012, 2013 p. 19, 20, 35 and data from the Agency for Food and Veterinary

There are dairies for collection, storage and processing of sheep's, cow's and aggregate milk in the confluence of the river Bregalnica, and mostly they are located in: Budinarci, Smojmirovo, Berovo, Robovo, Pehcevo, Delchevo, Vinica, Stip, Kocani and Sveti Nikole.

According to the data in Table 4, the total production of sheep milk in 1991 amounted to 60.285 million liters, while in 2013 that amount dropped to 36.5 million liters. The reduction is 23.785 million liters or 39.45%. The total average production of sheep milk for a period of 23 years is 46.384 million liters.

The total milk production per sheep milk in 1991 amounted to 40 liters, while in 2013 that amount increased to 71 liters. The increase is 31 liters per milk sheep or 77.50%. The total average milk production per milk sheep for a period of 23 years is 53 liters.

Production of sheep milk at the confluence of the river Bregalnica

According to the data in Table 4, the total production of sheep milk at the confluence of the river Bregalnica in 1991 is 12.672 million liters, while in 2013 that amount dropped to 7.539 million liters. The decrease is 5.113 million liters or 40.50%. The total average production of sheep milk for a period of 23 years is 10.663 million liters. The total milk

production per sheep milk in 1991 amounted to 37 liters, while in 2013 that amount increased to 69 liters. The increase is 32 liters per milk sheep or 86.48%. The total average milk production per sheep milk for a period of 23 years is 50 liters.

Conclusion

Considering the state above, it can be concluded that beside the fact that sheep and goat breeding are one of the most traditional and at the same time most economic livestock industries in the Republic of Macedonia, the existing capacities remain unsufficiently used. Also, an additional problem is the huge reduce of the pasture areas so the breeders are facing some serious issues. Other important facts to be mentioned are:

Total number of sheep from 1991 to 2013 is being decreased for 68%, but the production of sheep milk in this region has increased for significant 79%.

The percentual participation of the number of sheep at the confluence of the river Bregalnica in the Republic of Macedonia for the analyzed period is 21.75%.

The percentual participation of the number of goats at the confluence of the river Bregalnica in the Republic of Macedonia for the analyzed period is 27.36%.

The participation of sheep from the municipalities in the total number of the Republic of Macedonia is: Berovo 11.0%, Vinica 6.0%, Delcevo 5.0%, Zrnovci 0.5%, Karbinci 8.0%, Konce 2.0%, Kocani 3.0%, Kratovo 0.7%, Lozovo 4.0%, Makedonska Kamenica 2.5%, Pehcevo 6.0%, Probistip 10.0%, Radovis 1.0%, Sveti Nikole 17.0%, Cesinovo-Oblesevo 4.6%, and Stip 18.6%,

On the other hand, the participation of goats from the municipalities in the total number of the Republic of Macedonia is: Berovo 7.0%, Vinica 15.0%, Delcevo 10.0%, Zrnovci 2.0%, Karbinci 10.0%, Konce 2.0%, Kocani 10.0%, Kratovo 2.0%, Lozovo 2.2%, Makedonska Kamenica 9.0%, Pehchevo 2.0%, Probishtip 9.0%, Radovis 1.1%, Sveti Nikole 4.0%, Cesinovo-Oblesevo 3.0% and Stip 11.7%.

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EVALUATION OF LABOUR - EARNINGS - THE STRATEGIC ASPECTS OF THE PAYMENT OF HUMAN RESOURCES IN THE BUSINESS SYSTEM

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Abstract

In the paper public company is presented as an organizational system that is organized into three organizational levels. These are directorate of the enterprise, its branches and organizational units. Their tasks are: carrying out the development, coordination, control functions, the formation of profit centers and forming the basic unit of planning, organizing, and conducting expert technical jobs. Public company manages a total area of 130,000 ha, of which about 115,000 ha of forest, and one part is the rest of the land. In the structure of employed staff there are 15% (232) with university degree, with a college degree 2% (26), with secondary education 32% (503) and a lower 51% (799). Annual turnover of employees index is 1.28% and the stability index is 86.33%. Rewarding of employees is achieved by creating a stimulation system, which include salaries, benefits, rewards and other values that the employer provides to employees in exchange for their work. Reward system consists of tangible and intangible incentives. The system includes two forms of earnings: direct form of salary (basic salary, the increase in basic wages and salaries based on performance). Indirect benefits include the form of wages and benefits. The traditional approach to the determination of wages is based on the evaluation of operations, as the process that determines the relative value of jobs within an organization. The most commonly used method of assessment of the employees are: job ranking method, a method of classifying jobs and analytical job assessment (scoring method). Earnings of employees in observed public company is defined as follows: labor costs determined on the basis of the type of work and the importance of this work for the company; achieved performance; achieved norms and standards of work; incentives and disincentives and contribution of an employee when does not determine the individual effect of work. Points method is used when evaluating the jobs. Earnings based on performance in this accounting system does not apply, because there is the opinion among employees that the fixed salary they receive is quite satisfactory, although among the young people with higher education degree they are increasingly desirable. All these aspects are discussed in detail in this paper.

Key words: business system, wages - payment methods, forms of payment.

Introduction

The Public Company for forest management "Vojvodinašume" is analysed in the paper as organizational system whose elements are intended to carry out the development, coordination, control functions, the formation of profit centres and the basic units of planning, organizing and performing expert and technical activities.

In order to acquire a better picture of the reporting company, the insight into the total area of land managed by the company is given, and its general structure. Also, the structure of personnel employed is analysed by professional qualifications, as well as the annual index of turnover of employees and the stability index.

For a successful business operation of an enterprise, it is essential to attract and retain capable people, also to encourage and motivate the employees to develop their abilities, knowledge and skills, motivation, and creating a culture where employees care about the company in which they work. For this reason, special attention in the paper is devoted to the remuneration system, which achieves the harmonization of individual interests of the employees and the companies' strategic goals.

Materials and methods

To assess the severity of complexity, i.e. the value of work - labour, the ranking method, the method of classifying job positions and analytical assessment of tasks/work (points method), are mentioned as the most important. The annual index of turnover of employees is highlighted, as well as the stability index and the direct and indirect forms of the remuneration system. The systems of individual salaries based on the performance, systems of group remuneration and organizational rewards, as well as rewarding of managers are also presented. The literature sources as well as internal documentation of PC "Vojvodinašume" are used in dealing with the stated topics.

Results and discussion

Public Company for Forest Management "Vojvodinašume" is organized in three organizational levels:

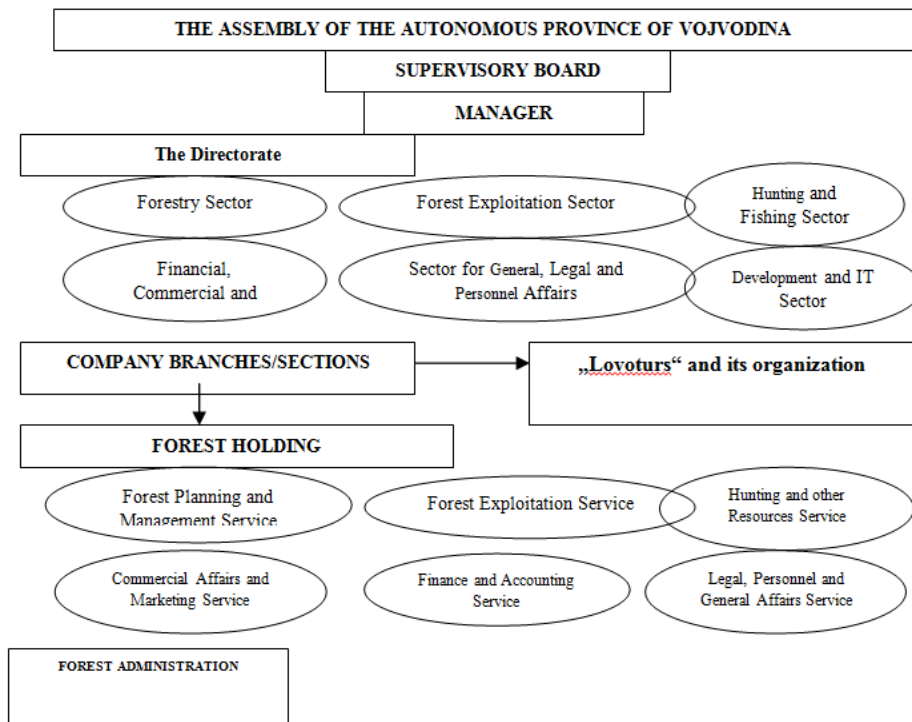
1. The Directorate of the company,
2. The Branches/Sections of the company - Forest holdings (4) and the section of the company "Vojvodinašume - LOVOTURS"
3. Organizational units: work units of forest administration (19) and other working units (5).

The organizational structure of the company is presented in the Diagram 1, while forest holdings and forest administrations that are integral part of the company are presented in the Diagram 2.

The Company Directorate performs development, coordination and control activities and deals with the strategy, development and coordination of the work and supervises the branches/sections of the company. The organizational structure of the Directorate consists of six sectors: Development and IT Sector, Sector of Forestry, Hunting and Fishery, Sector of Forestry Exploitation, the Financial Sector and Sector of Commercial Affairs and Marketing, Sector of General, Legal and Personnel Affairs. The bodies of the company are

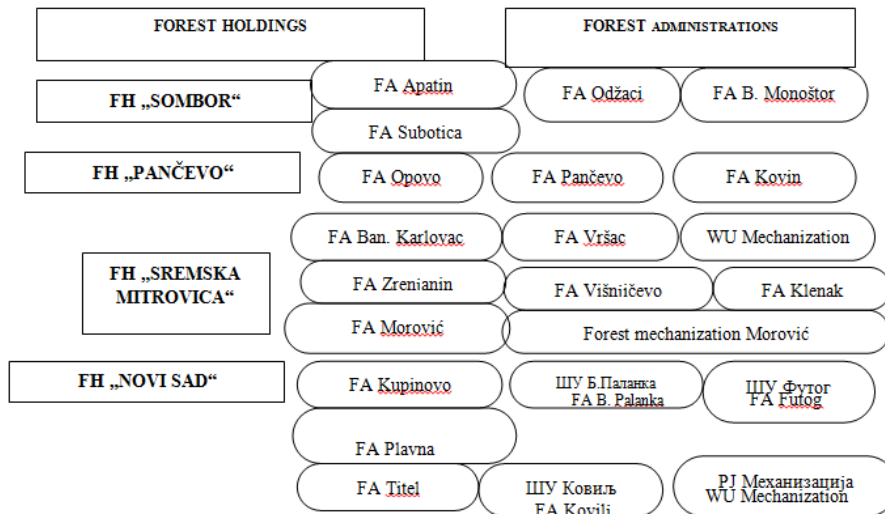
the Director/Manager and Supervisory board, appointed by the Government of the Autonomous Province of Vojvodina.

Diagram 1. Organisational scheme of the PC „Vojvodinašume“



Source: www.vojvodinasume.rs

Diagram 2. Forest holdings and forest administrations of the PC „Vojvodinašume“



Source: Author's interpretation

Forest holdings are formed at the level of forest areas and are profit centres and their organizational structure consists of services. Four forest holdings operate as a part of the Public Company "Vojvodinašume and a part of the PC "Vojvodinašume - LOVOTURS", with its organization specialized in hunting, hunting tourism and breeding of pheasants.

Forest administration units are the basic units of planning, organizing and conducting professional - technical activities related to forest management. Within four forest holdings there are a number of territorial forest administration units.

The estimated value of assets/property and equipment of PC "Vojvodinašume", in 2014 was 1,998,441,000 dinars, while the planned value for 2015 amounts to 2,373,428,000 dinars (Table 1).

Table 1. Forest funds of PC „Vojvodinašume“ of forest holdings in 2015

No.	Forest holding	Total area (ha)	Forests and forest land (ha)	Remaining land (ha)
1	Novi Sad	14.143,93	12.468,49	1.661,51
2	Pančevo	49.494,60	44.317,50	5.141,64
3	Sombor	23.594,97	18.669,95	4.907,14
4	Sremska Mitrovica	42.644,34	38.810,28	3.683,64
	Total PC „Vojvodinašume“	129.877,84	114.266,22	15.393,93

Source: Internal documents of PC „Vojvodinašume“

Public Company "Vojvodinašume" manages a total area of about 130,000 hectares, of which about 115,000 hectares are forests and forest land, and the remaining 15,000 hectares of land make up the rest. Complex business systems as a result of large capital investment can operate profitably and generate high profit rate on the capital invested only in conditions of the division of labour and management functions.

The management bodies of the PC "Vojvodinašume" are:

1. The Management Board of the company, which has 11 members, of which five members representing employees of the company;
2. The Executive Business Board of the company consisting of 12 members, six assistant directors headed by the Director of the company and five directors of the company branches/sections; and
3. The Supervisory Board of 3 members, appointed in accordance with the applicable Law on public enterprises (Table 2).

The structure of employees is as follows: the share of staff with university degree – the highest education is 15% (232), with higher education 2% (26), with high school education 32% (503), and lower education/qualification 51% (799). Forestry engineers make up 10.9% of employees (170) and forestry technicians 20.51% (320). The age structure of employees is relatively unfavourable, because currently there are no job positions open based on the natural outflow. The company employs 249 female workers (15.96%) and 1,311 (84.04%) males. Domination of male employees over the female originates from the activities of the company, predominantly hard physical labour, especially in direct production.

Table 2. The management structure of PC "Vojvodinašume"

No.	PC "Vojvodinašume"	Function
1	The Company Directorate	Director/Manager
	Forestry Sector	Assistant Director of Forestry
	Forest Exploitation Sector	Assistant Director of Forest Exploitation
	Hunting and Fishing Sector	Assistant Director of Hunting and Fishing
	Financial, Commercial and Marketing Sector	Financial, Commercial and Marketing Assistant Director
	Sector for General, Legal and Personnel Affairs	Assistant director of General, Legal and Personnel Affairs
	Development and IT Sector	Assistant Director of Development and IT
2	Company Branches/Sections	
	Forest holding "Sombor" Sombor	Branch/Section Director
	Forest holding "Banat" Pančevo	Branch/Section Director
	Forest holding "Sremska Mitrovica" Sremska Mitrovica	Branch/Section Director
	Forest holding "Novi Sad" Novi Sad	Branch/Section Director
	"Vojvodinašume - Lovoturs" Petrovaradin	Branch/Section Director

Source: Internal documents of PC „Vojvodinašume“

Annual index of employee turnover or rate of reduction in the number of employees as a percentage in the PC "Vojvodinašume" in 2015 was 1.28% and the decrease mostly related to retirement and termination of the specified time contracts. This indicator does not give an objective amount of information, because it does not take into account the length of service, or information about specific job positions that are being vacated. Stability Index is based on the number of persons who were able to remain at work for a period of time, and for this company in 2015 it was 86.33%, meaning that over 86% of people have remained at work for a certain period, and that there is a certain continuity in employment, which is favourable from the viewpoint of stability of the company, as there is no leaving of the organization.

System of earnings (wages) and strategic aspects of payment

The principle of remuneration is achieved by creating a system of motivating employees which implies salaries, fees, rewards and other items of value that the employer provides to employees in exchange for the work. The main role of the remuneration system is to harmonize the interests of individual employees and strategic corporate objectives by attracting and retaining the right people, encouraging and supporting employees to develop their abilities, knowledge and skills, motivation, and creating a culture in which employees care about the company in which they work.

The remuneration system comprises tangible/material and intangible/non-material compensation and incentives.

Salary system comprises two types of earnings:

1. Direct forms of earnings comprise elements of earnings which employees receive in cash: basic salary, increase of the basic salary to maintain the purchasing power and earnings that depend on performance.
2. Indirect forms of earnings include benefits. Benefits for employees and their families provide security, and include two types of programs:

a) Protection programs for employees: health insurance, pension insurance, disability insurance, life insurance, etc.

b) Paid leave: military service, vacations, sick leave, maternity leave, etc.

The benefits represent indirect earnings that are related to the status and status features and include various forms, such as free meals or meals at subsidized prices, the use of a company car, free recreation, the use of a mobile phone, etc.

The structure of the basic salary can be defined in two principal ways:

1. The application of the traditional approach, which is basically the assessment of job positions, it is dominant and based on assumptions of the theory of justice or equality in anticipation of appropriate remuneration for their labour input. The traditional approach relies on the evaluation of activities which determines the relative value of job positions on the basis of predetermined criteria such as the required education, experience, skills, level of responsibility for a given job, the effort and the characteristics of the conditions in which the work is performed. The most commonly used methods of evaluation of the job positions are:

- Method of ranking job positions - the oldest and simplest method in which each employee is compared with each other and at the same time the superiority is established. It is not suitable for large organizations or for organizations without a clearly defined hierarchical structure.
- Method of classifying job positions - by which one must first determine the scale of values with a detailed definition of what each level means. Comparison is done by observing entity as a whole. This method is not viable for measurement of group of employees.
- Analytical assessment of job positions (points method) - is based on a comparison of job positions based on common factors which "carry" a different number of points, where the number of points depends on the significance of certain factor. The advantage of the method is that the number of points indicates the relative differences between job positions in quantitative indicators.

PC "Vojvodinašume" has a very complex system of earnings/wages. For calculation of salaries, employee in the Sector of Finance, Commercial Affairs and Marketing uses computer package "AB SOFT", which facilitates and speeds up the process.

Basic elements of earnings (salaries) for one employee are:

1. The salary received by the employee for work performed and time spent at work,
2. Increased earnings,
3. The wages and other remuneration, other than reimbursement of expenses, and
4. Other income, which by law are not considered earnings.

Earnings of employees in PC "Vojvodinašume" are defined as follows:

- Price of labour, determined on the basis of the type of work and the importance of this work for the company,
- Performance achieved,
- Set standards and labour standards, which include the assessment of the employer on the quality of work performed,
- Incentives - disincentives,
- The contribution of the employee, when not determined by individual performance.

In determining the structure and level of earnings/wages or salaries earned by the employee for work performed and time spent at work, the company uses the traditional

approach, which relies on the evaluation of job positions on the basis of predetermined criteria, such as the skills, effort, required education, work experience, etc.

The method of points is used for evaluation of job positions. Price of labour is the amount that is agreed in the Employment contract and cannot be lower than the price of labour for the typical activities and is expressed in points. Each job is valued by allocating corresponding number of points based on the complexity, the required education, responsibility and working conditions of a particular workplace. Ranking and control of the performed assessment of tasks or job positions, is done in a way that all the results of the evaluation are ranked according to the number of points, starting from those with the least, to job positions with the highest number of points. Subsequently, the complexity of certain workplaces or job positions is compared, from the standpoint of the number of points and possible discrepancies are determined in relation to other job positions. In the event of established discrepancies, control and reassessment of that job position or the workplace is performed.

The value of one point is determined in the way that the sum of money allocated for the company's monthly salary is divided by the number of points for all job positions in the company for that month. The job or workplace is estimated in the following way: the number of points for the specific job is multiplied by the value of one point, to obtain the value of the complexity of the job position or workplace. The value of the point for the month, for which payment is made on the basis of business results/performance, is determined by the Director for the Directorate and Directors of the Company Sectors for the Sectors of the company. The fixed monthly salary of an employee is calculated by converting starting points in accounting/calculation points, and the value of one point is 19.34 dinars. Table 3 shows the number of points allocated for different workplaces/job positions in PC "Vojvodinašume". The increase or reduction of basic earnings based on the quality and the scope/volume of work (**incentives or disincentives**) is determined by the decision of the Director or an authorized person and can range from +20 to -20% of the basic salary. An employee is entitled to the increase of the basic salary for the following:

1. Seniority. The basic salary of an employee is increased in the amount of 0.5% of the price of labour, for each full year of service, plus the insurance period to be calculated at an accelerated rate (past performance). The right to early retirement options have cutters (1 year and 3 months, i.e. 5.5% increase of basic salary) and drivers (2 months i.e. 3.5% increase of basic salary)
2. Overtime. The increase of earnings per working hour - for work longer than full time (overtime) not including the working hours pre-determined on the basis of the rescheduling of working hours by 30%.
3. Work on holidays, which are non-working days. For work on the days of national and religious holidays which are determined according to the law as non-working days earnings is increased by 100%.
4. Night work. For work at night between 10 pm and 6 am of the next day earnings are increased by 30%.
5. Work in shifts.
6. Hot meal. Reimbursement of costs of nutrition (meal) during work is provided in the amount of 20% of average monthly salary in Serbia's economy.
7. Field work allowance. Daily remuneration for the increased cost of labour and field work is provided in the amount of 3% of the average monthly salary in Serbia's

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economy, and the accommodation shall be provided for the duration of field work. The employees are eligible for this benefit if they perform work in the field for more than 5 days outside the headquarters of the company.

8. Pay for annual leave. Employees are eligible for the reimbursement of costs of annual leave (holiday), provided that they are entitled to the annual leave of at least 18 days, in the amount of average monthly salary in the economy of the Republic of Serbia, i.e. the proportional share if the employees are entitled to less than 18 working days of annual leave. If employees work on Sundays, a day-off shall be provided to them during the next week.

Table 3. Table of points for job positions at JP "Vojvodinašume"

No.	Job titles	Labour cost in points
	I GROUP: jobs requiring low qualification (LQ)	
1.	Cleaner, courier, cafe cook, custodian, assistant cook, coachman and other jobs within the group	1000 - 1500
	II GROUP: II level	
	<i>Competence acquired from 6 months to 2 years</i>	
2.	Forest worker, fish warden/keeper, host of the hunting house and other jobs within the group	1500 - 2000
	III GROUP: III level (jobs requiring qualification) (Q)	
3.	Forest worker - cutter, motor vehicle driver, operator of working machines, seller, cook, waiter, handyman maintenance, warehouse worker and other jobs within the group	2000 - 2500 (Forest worker - cutter - 2800)
	IV GROUP: IV level (jobs requiring high school education) (SE)	
	<i>The competence acquired through secondary education lasting 4 years</i>	
	Manager/foreman (Q - SE), forest ranger, game warden/keeper (Q - SE), accountant, treasurer, administrative worker and other jobs within the group	2000 - 2800
4.	V GROUP: (HQ)	
	<i>The competence acquired by specialization on the basis of secondary education</i>	
5.	Head of the restaurant/catering facility, accountant and other jobs within the group	2800 - 3000
	VI GROUP: VI level (HE)	
	<i>The competence acquired by higher education</i>	
6.	Chief of the accounting, officer for surveying and other tasks within the group	3000 - 4000
	VII GROUP: VII level (HE)	
	<i>University degree, Faculty education</i>	
7.	Head of the Forest Administration unit, head of the department and division, the hunting ground manager, an engineer, an independent officer, clerk, designer and other jobs within the group	4000 - 5800
	VIII GROUP: VII level	
	<i>Requires competence acquired through a master degree or specialization on the basis of faculty education</i>	
8.	Research – assistant, research associate, etc.	4200 - 5000
	IX GROUP: VIII level	
	<i>Requires competence acquired through doctorate - PhD</i>	
9.	Principal research fellow, senior research associate, research associate and other jobs within the group	5000 - 6000

Source: Internal documents of PC „Vojvodinašume“

Payment of wages (salaries) to all employees of PC "Vojvodinašume" is carried out twice a month, exclusively in cash, through bank accounts of the employees. The planned average monthly gross salary per employee, in 2015, is 70,642 RSD.

The fund for employees' salaries for 2015 is planned in the amount of 1,326,807,119 RSD, which does not exceed 2% increase compared to the planned amount in 2014. The planned fund for employees' salaries (gross) in 2015 accounted for 30.21% of the total income of the company, and 29.37% of the total expenditure.

Earnings based on performance

In contrast to the basic salary, which is determined based on the value of work performed by an employee, earnings based on performance achieved are determined according to the value of the contribution of individuals, teams or organizational units. Linking earnings with productivity, implies that the salary costs are associated with the achieved results. The best performers of specific work invest extra effort into the increase of the productivity, when they are aware that it would lead to obtaining higher earnings. Earnings based on actual performance may be individual, group or organizational.

- The individual rewarding system is used when the individual productivity is measurable and can be created according to individual preferences. The systems of individual performance based earnings in practice are following:
 - Payment system per unit of production is based on the payment of employees at a fixed rate for each unit of production. This payment system is difficult to use, because for some tasks it is difficult to determine standards.
 - Commission fees are individual remuneration system that is widely used in sales and is calculated as a percentage of the cash amount of the total realized sales. Payment on a commission basis represents an effective financial reward, and can reduce the teamwork, because employees can compete with one another.
 - The bonuses represent one-off payments, which are given for achieving an organization's goals. Bonuses are calculated on an annual basis, as a percentage of the basic salary of an individual (the usual rate is 5%).
- The system of group remuneration for realized performance is based on determining the size of the earnings on the basis of group performance and the results achieved at the level of the team, organizational units or entire enterprises. A decisive factor in the system of group remuneration is the size of the group.
- Organizational rewards are given to all employees in an organization on the basis of business success during the year. The purpose of these rewards is to encourage teamwork. The most famous systems that provide organizational rewards are:
 - Scannell's plan, which includes the participation of employees to reduce labour costs. The savings arising from differences between actual and expected proportion go into the fund used to pay bonuses. Further funds are distributed according to a predetermined proportion. A typical distribution of savings is as follows: 50% for employees, 25% for employers and 25% for the fund for contingencies.
 - Plans for a share of the profits are intended to focus the awareness of employees on profit, to encourage cooperation and teamwork and that the employees are involved in the success and growth of the organization. The common plan for a share of the profits is a plan that allows employees to buy shares in their company, thereby gaining ownership of the company in which they work, thus reinforcing their commitment,

loyalty and motivation, and companies are exempted from tax for dividends paid to employees. The disadvantage of this plan is that the salaries of employees depend on the business results of the company.

Public Company "Vojvodinašume" in its accounting system does not apply performance based earnings system. The opinion among employees is that the fixed salary they receive is quite satisfactory, independent of their personal performance.

However, performance based salaries are becoming increasingly popular among young people of higher education and high performances.

Rewarding managers

Identification and evaluation of the contribution of managers is a very difficult and complex process. It is customary that the reward for managers consists of:

1. Basic remuneration (salary);
2. Bonus (short and long term);
3. Various benefits and privileges (regular and special).

In addition to these elements of rewarding managers, there is another component of the earnings representing the status feature, and these are the following benefits:

1. Benefits related to the work of a manager in the office;
2. Benefits related to the work of managers outside the company;
3. Personal benefits of the managers.

Considering that the points system is used in calculation of salaries for all management jobs in the PC "Vojvodinašume", additional points are established in the calculation of earnings. Depending on the work being performed and the status and the position in the company, employees who perform management duties, will have the basic salary increased by 100 to 2200 points. Earnings of the management bodies are earnings of company directors, assistant directors and directors of the company sectors/divisions, and the employees performing these functions receive increased salary by a certain number of points.

Monitoring individual work results

Performance assessment through general assessment of persons from living and working environment, which entails corresponding social and economic consequences, is a serious, complex and responsible job. The problem occurs in larger companies where those who manage these processes lack an immediate, reliable access and insight into the individual results of employees. The basis for establishing success criteria are job position and job analysis, and the corresponding factors. The subject of assessment can be:

- The personality of the individual as a whole;
- Abilities and personal characteristics of individuals;
- The results of the work;
- Characteristics of organizational behaviour;
- Objectives and expected results;
- Potential of collaborators.

Assessment and monitoring individual performance of personnel is done by using different methods, and in practice the most commonly used are: the evaluation scale, numerical scale, descriptive scale and the scale of judgements. Whatever method is applied, it is necessary to decide which will be used as a source of information on the

performance of employees. Assessing and monitoring the results of the work may be carried out by: 1. managers, 2. colleagues, 3. subordinates, 4. self-assessment, 5 clients. Successful managers provide feedback to their employees on their (work performance), lack thereof and individual results, in a way that encourages positive behaviour.

Evaluation of the operation and performance of employees in PC "Vojvodinašume" is one of the most important tasks. It shows the success of the selection, training and coaching and the guidelines for further planning of personnel development, of the system of rewarding and discipline. In order to clearly define the objectives and criteria of work, the manager and employee establish a well-formed network of information flow. The aim of the company is that the monitoring of the results and performance assessment are useful, and that the work of the employees is regularly and frequently monitored, because the final evaluation at the end of the year is not enough.

For most managers of PC "Vojvodinašume" evaluation and monitoring of the individual results of the work is unpleasant activity, and they would like to avoid it. The main reason for this is poor preparation for the interview, and inadequate communication about the results, which does not encourage employees to eliminate errors and improve their performance.

It is very important to understand what is the purpose of monitoring and evaluation of the work and results of employees, and what kind of benefits the employee and the manager of the company can achieve, namely: improvement of the performance, timely feedback, building of self-esteem and increase of the work efficiency.

Conclusions

The paper describes the role of the rewarding system, and the system of earnings in the particular company and points to the strategic aspects of payment. The elements of salaries – earnings are indicated and the level of basic earnings specified, also earnings that are based on performance (short term, long term, individual and group). It is pointed out that the system of rewarding includes money, goods, status, services that the employer provides to the employees in exchange for the work. Reference is made to direct forms of earnings comprising elements of earnings that employees receive, in cash and in the form of indirect earnings that include benefits and advantages.

Emphasis is put on the main elements of salary, such as: basic price of labour, allowances, benefits, premiums, overtime, bonuses and incentives. An important component of the total salary of the employee can be a part of their earnings, which depends on the actual performance, which is determined based on the value and contribution of an individual, group (team), or organizational unit in the PC "Vojvodinašume".

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ECONOMICS OF AGRICULTURAL PRODUCTION IN IRRIGATION CONDITIONS ON THE AREA OF REGIONAL HYDROSYSTEM DANUBE-TISA-DANUBE

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Abstract

This paper examines the economics of agricultural production under irrigation. In case of local irrigation system in the area of 4000 ha, supplied with water from the canal Hs Danube-Tisa-Danube, production and economic effects of irrigation in crop, vegetable, fruit, forage crops, as well as double cropping system were analyzed. Economic indicators (total revenue, costs, profit) for the selection of production structure that can afford the costs of irrigation were analyzed for 22 crops. Seed crops and vegetables have achieved the best economic results in irrigation conditions in this local irrigation system. Fruit production did not achieve significant results. Studies have shown that the irrigation have significant impact on the cost of certain production (local system + price of water at the water intake). The price of water in the old system of irrigation was 10.53 din/m³, while in the new it is 27.31 din/m³. Fees for irrigation payable regional hydrosystem Danube-Tisa-Danube were 1779 din/ha for field crops and 2348 din/ha for vegetable and fruit. The method of irrigation cost allocation to the crops also has an impact on the operating results of the crop (monomial or binomial tariff). Cost-effective production management in irrigation calculate with the amount of product (in kilograms) in certain crops which have to be achieved and set aside to cover the cost of irrigation. This distinction is necessary to achieve to justify irrigation. For example, to cover the cost of irrigation with corn seed is necessary to achieve from 200-300 kg/ha with a green beans from 1000 to 2300 kg/ha.

Key words: production and economic effects, irrigation, costs, fees.

Introduction

The role of hydrosystem Danube-Tisa-Danube has undoubtedly been off essential importance in the economic development of the Vojvodina Province, and especially its agriculture. In this example as well has been confirmed that the lower costs of construction and exploitation are achieved by building the multi-purpose systems (protection against flood, drainage, irrigation, navigation, water protection).

The effects of irrigation (production and economy) were tested on surfaces that supply water to this hydrosystem (Potkonjak et al., 2013). It has been concluded that the capacity of the hydrosystem is still not used enough for irrigation because there is a lack of

investments in the local systems (both for small ones and large ones). The examination has also shown that the existing local water systems were not used enough even though there were several consecutive years of drought (Potkonjak *et al.*, 2013). The reasons for insufficient usage of capacity of hydrosystem for irrigation according to the research (Potkonjak *et al.*, 2011) are numerous: costs of irrigation, financing conditions of the construction of local systems, costs of agricultural products, lack of competitiveness and others.

Considering the present results, the objective of this paper was to analyze the usage and payment of the fees for irrigation in the municipalities in Vojvodina whose holding are supplied with water from facilities Hs DTD. They also analyzed production and economic effects of irrigation on a representative holding of agricultural area of about 4000 ha, which is supplied with water from the channel.

Materials and methods

Data for this survey were collected in the Public Water Enterprise (PWE) "Water Vojvodine", Novi Sad and on farm "Sava Kovacevic" in Vrbas. Data about irrigation point use was collected for regional hydrosystem Danube-Tisa-Danube. It also demonstrates the methodology of payment fees for irrigation by agricultural holdings for this hydrosystem. Big agricultural holdings, which have had irrigation system and water-source from channel DTD for several years were chosen. Based on the records kept on the farm, calculations of the effects of irrigation were made using standard econometric methods and techniques for measuring the effect.

Results and discussion

The price of water for irrigation

The water price in local irrigation systems in Vojvodina is specific for each system and depends on several factors. The water source (natural or artificial) has a significant impact on the price of one cubic meter water. In the case of regional systems, in the example of a DTD fee for irrigation paid to this system is calculated according to the prescribed methodology (Official paper of RS, no. 15/2015) and consists of:

The fees for irrigation Hs DTD = fee for the usage of water for irrigation + fees for usage of facilities

The fees for water supply depend on whether the device for measuring the amount of water is used or not. In the case of being used the fees are 0.1132 RSD/m³ or 679.1678 RSD/ha if the device does not exist.

The fees for the usage of Hs Danube-Tisa-Danube are paid basing on the installed and engaged capacity. In the installed capacity the fee for irrigation of surface under agricultural crops amounts 235.46 RSD/ha. Also, for irrigated land under vegetable crops, orchards and perennial plants it is paid 327.54 RSD/ha. If it is a case of engaged capacity and the taxpayer has measuring device, the fee is 724.54 RSD/1000 m³. In the end of the fee payer who does not have a measuring device for irrigation of land under agricultural crops, it is paid 833.20 RSD/ha and for irrigated land under vegetable crops, orchards and perennial plants it amounts 1341.41 RSD/ha.

Summing these two types of fees, total fee that fee payer pays to Hs DTD amounts, 1779 RSD/ha for crops and 2348 RSD/ha for vegetable crops and perennial plants (without

Table 1 shows the structure of crop production on the holding (average 22 crops per year) and yields that have been achieved in the case of irrigation (kg/ha). Present economic indicators are: the selling price of the product, the value, the costs of production and financial results (profit or loss). In Figure 2, the profits earned in 2014 year by individual crops are presented. It can be noted that the seed crops and fruit achieve the highest unit profit (RSD/ha). The average course of EUR in 2014 was 119 RSD.

Table 1. Effects of agricultural production in irrigation

No	Crops	Yield, kg/ha	Selling price, din/kg	Production value, din/ha	Production costs, din/ha	Financial results, din/ha
1	Wheat for seed	6740	27.72	186832.8	113805	73027.8
2	Wheat	5417	17.59	95285.03	94139	1146.03
3	Maize for seed	2690	130	349700	192440	157260
4	Corn for grain	6547	17	111299	100984	10315
5	Sugar beet	47891	4.73	226524.43	180309	46215.43
6	Soybean	2564	50.19	128687.16	108300	20387.16
7	Silo maize	46487	4	185948	174066	11882
8	Pease	4592	27.04	124167.68	102864	21303.68
9	Dill	15186	8	121488	107923	13565
10	French beans	5238	25	130950	116692	14258
11	Parsley	31135	10.28	320067.8	222920	97147.8
12	Parsnip	20985	10.27	215515.95	153459	62056.95
13	Lucerne for hay	13671	15	205065	125917	79148
14	Plum	19500	18	351000	234000	117000
15	Apple	23490	25	587250	399330	187920
16	Stubble corn for seed	2348	131.54	308855.92	110801	198054.92
17	Stubble sweet corn	3645	25	91125	81222	9903
18	Stubble green beans	8269	18.46	152645.74	113242	39403.74
19	Stubble millet	30518	4	122072	106771	15301
20	Stubble silo maize	23048	3.5	80668	76416	4252
21	Stubble spinach	9977	20	199540	144687	54853
22	Stubble sunflower	1816	45.6	82809.6	64010	18799.6

Holding's irrigation systems (old and new) had had an impact on improving the economic effects during recent years in certain cultures and had caused significant exploiting costs. To monitor these costs (fixed and variable) additional calculations have been done. The price of 1 m³ of water in the old system amounted to RSD 10.53 / m³ (variable costs amounted to 4.68 and 5.85 fixed RSD/m³). The cost of 1 m³ of water on the new system amounted to RSD 27.31 / m³ (variable costs amounted to 3.73 and fixed 23.58 RSD/m³). The reason for the significantly higher price of a cubic meter of water in the new system was the interest on loans, which are fixed costs of irrigation, representing 42.95%. After repaying the loan, the reduction of cost for irrigation can be expected for the new system.

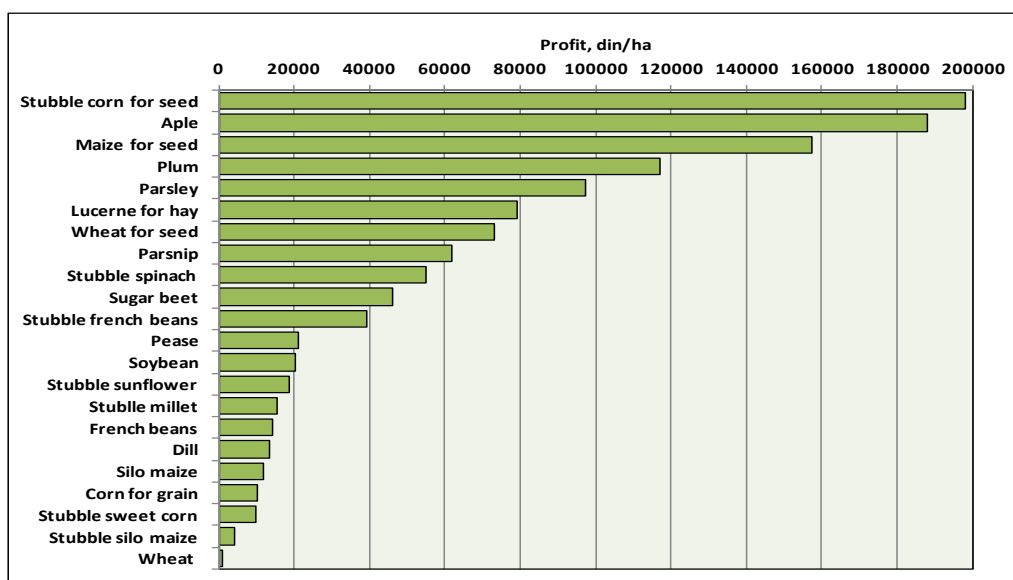


Figure 2. Achived profit by individual crops (in 2014. year)

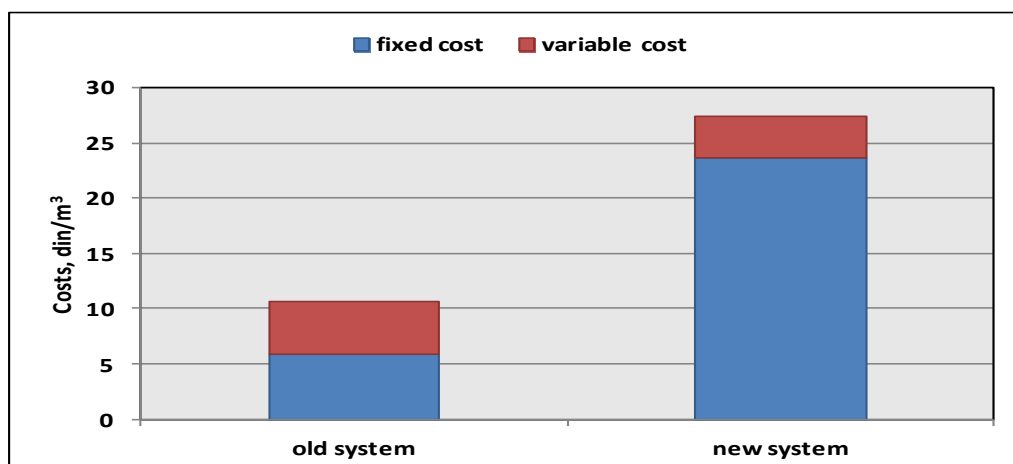


Figure 3. Partake of fixed and variable cost

In Figure 3 the behavior of the fixed and variable costs in the old and new irrigation systems is displayed.

On the basis of the data on consumption of water by individual cultures irrigated in 2014, as well as the ratio of fixed and variable costs and cost (culture) required amount of products necessary to cover the cost of irrigation for the old and new system was calculated (Tables 2 and 3). In other words, this quantity of products is needed to achieve an additional yield of each culture in order to cover the costs for irrigation. Results show that in most crops this difference is not difficult to achieve.

SECTION 2. AGRICULTURAL ECONOMICS

Table 2. Needed quantity of agricultural product for cover of irrigation cost (old system)

No.	Crops	Water consumption, m ³ /ha	Yield, kg/ha	Price, din/kg	Irrigation costs, din/ha	Needed, kg/ha
1	Maize for seed	1581	2690	130	26078	200.60
2	Sugar beet	1396.	47891	4.73	23021	4867.02
3	Soybean	1716	2565	50.19	28303	563.93
4	Silo maize	1930	46487	3.5	31831	9094.57
5	Corn for grain	1875	6547	17	30916	1818.59
6	Pease	338	4593	27.04	5574	206.14
7	Parsley	3100	31135	10.3	51114	4962.61
8	Stubble corn for seed	1600	2349	131.5	26380	200.60
9	Stubble sweet corn	3200	3645	13.7	52760	3851.12
10	Stubble green beans	1150	8269	18.9	18960	1003.21
11	Stublle millet	1842	30518	3.6	30384	8440.08
12	Stubble silo maize	500	23049	3.5	8243	2355.37
13	Stubble spinach	1300	9977	8	21433	2679.23
14	Stubble sunflower t	554	1817	45.6	9147	200.61

Table 3. Needed quantity of agricultural product for cover of irrigation cost (new system)

No.	Crops	Water consumption, m ³ /ha	Yield, kg/ha	Price, din/kg ,	Irrigation costs, din/ha	Needed, kg/ha
1	Maize for seed	1850	2690	130	41165	317
2	Sugar beet	573	47891	4.73	12753	2696
3	Soybean	1700	2565	50.19	37839	754
6	Pease	729	4593	27.04	16238	600
7	Dill	100	15186	8	8110	1014
8	French beans	1190	5237	20.5	26476	1291
10	Parsnip	1539	20985	10.3	34263	3326
11	Plum	500	19520	21.69	11134	513
12	Apple	1285	23490	31	28605	916
13	Stubble corn for seed	1929	2349	131.5	42938	326
15	Stubble green beans	1968	8269	18.9	43801	2317
16	Stublle millet	1432	30518	3.6	31868	8852
18	Stubble spinach	2125	9977	8	47278	5910

Conclusions

The research results show that holdings which operate under irrigation achieve better economic and financial effects, especially if they are supplied with water from the regional system. To confirm this research it is essential to keep records of effects as well as to calculate costs of the irrigation system. It is necessary to work on improvement of the methodology of cost allocation of irrigation to the carriers (cultures). In the case of water consumption for irrigation by individual cultures, usage of contemporary methods for determining the annual irrigation necessity for a certain culture is recommended. The

effects of irrigation should be also expanded to better usage of other factors of production (machinery, labor, processing capacities, market) and not only on increase of yield and income.

Acknowledgements

The work was undertaken as a part of the project no. 46006: *“Sustainable agriculture and rural development in the function of accomplishing strategic objectives of the Republic of Serbia in the Danube region”*. It has been funded partially by the Ministry of Science, Technology and Development, Government of Serbia.

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THE LEAN CONCEPT AS THE SOURCE FOR IMPROVING ECONOMIC PERFORMANCES IN AGRIBUSINESS SECTOR

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Abstract

The *Lean* production concept represents an approach that adds value for customers through identifying and eliminating waste in production within the whole supply chain. That means it is attaining more with less (resources). In other words, the *Lean* goal has been realized by eliminating all those factors which consume resources, efforts and time but not adding the value. Further, this results in the continuous improvement of productivity growth, cost reduction, efficiency and profitability increase. The recent experiences in the *Lean* concept implementation indicate that listed effects can be attained without significant investments, i.e. the effects have been achieved not by more complex operation performance but by the more intelligent one (to work smarter, not harder). In the research and analysis there were used several methodological procedures, such as the desk research method, the methods of analysis and synthesis, deduction method and methods of descriptive statistics. The potential of *Lean* idea can be successfully implemented in all sectors, including the agribusiness sector. Therefore the *Lean* concept is realized as an important element for improving the competitiveness of agricultural producers and positioning their products at the global market. In the region of the South-East Europe the *Lean* concept has not been largely represented. The goal of this paper is to highlight the potentials and limits of the *Lean* concept in agribusiness sector.

Key words: *Lean* concept, agribusiness, added value, competitiveness, sustainability.

Introduction

Numerous enterprises and subjects in the field of agribusiness, including farmers, use the *Lean* system in order to maximize business efficiency. This is achieved through the most efficient use of production resources as well as reduction of waste, thus providing sustainable production. No less important is the need to be increased the morale and thus productivity of employees, in order to be realized the *Lean* system potentials as much as possible. Experiences in this regard indicate that the volume of production can be increased even by 50% under other unchanged conditions. At the same time, an increase of productivity refers not only to the physical work but also to the change in awareness of employees in the sense that application of the *Lean* system allows employees to have a better control of the working process and it initiates a higher degree of self-control.

Besides the production and process part of the business operation, application of the *Lean* principle provides more efficient coordination in the field of logistics (transport and

storage), but also a more efficient debt collection for the goods delivered, which is a key assumption for adequate planning and maintaining the liquidity of the enterprise in the short, medium and long term. This suggests that the introduction of the *Lean* concept contributes that all activities of the enterprise should be planned, controlled and measured. This not only provides a high level of customers' satisfaction but also a competitive advantage based on the cost leadership. As the result of mentioned effects, there have been significantly improved the economic performances of enterprises in the conditions when it has been applied the *Lean* system compared to the traditional production condition.

A number of authors in the countries where the *Lean* idea has been widely accepted for decades, have studied the achievements and effects of this concept in different areas of production and services. Staats and Upton (2008) wrote about the *Lean* principles and learning, Moreira et al. (2010) have been explored eco-efficient of *Lean* production systems, Silva et al. (2011) wrote about factors for successful implementation of the *Lean* manufacturing, Distelhorst et al. (2015) explored *Lean* effect on labor standard. When we talk about studies of the *Lean* concept in domain of agribusiness sector, we must point out on Lehtinen and Torkko (2005), who explored the *Lean* implementation in food industry, Zokaei and Simons (2006) who explored performance improvements through the *Lean* practices in meat industry, etc.

Because the *Lean* concept is not widespread in the region of Southeast Europe, the paper points out the potential and scope of *Lean* idea just with an aim to promote the management practices in the agribusiness sector and consequently to improve overall business results.

Materials and methods

In this paper it was used mostly foreign relevant literature, the secondary results of case studies and resources available on the Internet. The available data were analyzed using the "desk research" method.

Results and discussion

The Lean production concept

The *Lean* is a system that creates outputs' using less of every input and simultaneously offering an increased choice for the customers. The cornerstones of *Lean* are management of process and the integrated logistic flow, management of relationships with employees, teams, and suppliers, and management of the change from traditional production concept (Lehtinen and Torkko, 2005).

The *Lean* is currently one of the most potent production strategies on a global scale. Originally conceived and applied at the car factory Toyota, and wider accepted thanks to the excellent results that occur based on the effective implementation of the *Lean* concept. More precisely, the *Lean* provides high productivity, high level of production diversification as well as synchronization of production and demand. Achieving of the above cited objectives requires the use of the following five principles (Pascal, 2007):

- 1) Creating value for customers;
- 2) Identification of value streams. Considering and understanding of material and information streams that arise in the movement of products through the value stream;

- 3) Creating of flow;
- 4) The production of products that are attractive to customers;
- 5) Continuously identifying and eliminating inefficiencies in production.

The *Lean* concept represents an approach that adds value for customers, which represents one of the key factors for attracting and retaining them. It is also a necessary condition for the acquisition and increase of the market share. Hence, it is important to identify what it is (resources and activities) that add value to the products and makes them more attractive to the buyers. Through identifying and eliminating inefficiencies as well as the occurrence of waste in production at the enterprise level but also in the entire supply chain, the enterprise aims to achieve more with less (George, 2010). In other words, the *Lean* goal is realized by eliminating all factors that consume resources, the efforts of employees as well as the time, while at the same time they do not add value to the products. It further requires continuous reviewing and achieving improvements, which finally should result in growth of productivity, an increase of efficiency and profitability.

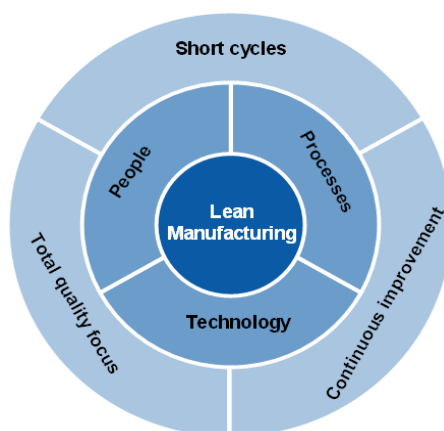


Figure 1. Lean manufacturing

Source: http://www.gallus-group.com/it/desktopdefault.aspx/tabid-318/473_read-910/

Previous experience in applying the *Lean* concepts suggests that these effects can be achieved without significant investments, which is being implemented by more intelligent operations and not by the complex operations. More precisely, we are not talking about the application of certain specific technological solution (technology is important but it is not always the solution for all problems), but the focus should primarily be on the idea that changes awareness and culture in a particular working environment by making people a key element of the production system (see Figure 1).

Potentials of *Lean* idea can be successfully implemented in all sectors, including the agribusiness one. Hence the implementation of the *Lean* concept is seen as an important factor of the competitiveness improvement not only of the agricultural producers, but also of other participants in the agribusiness sector, as well as the successful positioning of their products in the global market.

The factors that are the most important for the successful implementation of the *Lean* concept include the following (Womack and Jones, 2013):

- 1) Support of top management to the *Lean* idea and its implementation in the enterprise;

- 2) Focusing on goals (mapping the current state and the desired state, and identifying tools by which to achieve the objectives);
- 3) Long-term orientation;
- 4) Involvement of all stakeholders at all organization levels;
Involving of customers and suppliers (supply chain).

Application of Lean concepts in agriculture

Historically the *Lean* concept was originally implemented in the conditions of mass production (e.g. car industry). This type of production is typical for the agribusiness sector as well, why the *Lean* concept could be also successfully implemented in this sector.

Experience shows that the introduction and application of the *Lean* concept, the production volume can be increased up to 50% with other unchanged conditions. In addition, there could be significantly improved the product quality, the costs could be halved, the inventory turnover could be increased, delivery time can be reduced and finally the high profitability could be achieved (Black, 2008).

Previous experience also suggests that when farmers are involved in the supply chain, their participation in the *Lean* program resulted in an increased interest in acquiring new knowledge. As it has been already pointed out, in application of the *Lean* principles the emphasis is on the human factor. They come to the fore ideas on how to implement the operations in the best way. This is because it is necessary timely to bring many non-standardized decisions that are dependent on external factors. In particular, it is known that agricultural production to a large extent dependent on climatic conditions. Weather conditions vary between different geographical areas. In some areas it is prevailing drought in a certain part of the year, while the others have rainy weather and problems with flooding. So, there is no universal model that could be applied to all enterprises from the agribusiness sector. Also, the price volatility of agricultural products in certain economies imposes an additional challenge for agribusiness sector. It also has to be added the expressed discontinuity in agricultural policy conducting, changes of subsidizing conditions, the instability of the monetary unit, etc. Hence the organization of production according to the *Lean* concept has to be adapted to each enterprise, its specificities, the business conditions, the natural environment and other factors. It is important that there is a focus on the solving of problems, understanding their causes and finding adequate solutions for minimization of probability in their future repetition.

What is characteristic for the *Lean* concept is that production is oriented toward customers. Concerning agribusiness sector in the region of Southeastern Europe, it could be said that, with certain exceptions, generally there is no the same orientation. The production is mostly carrying out without adequate plan. Significant space for improvement exists in the area of quality improvement, an increase of environmental protection, improvement of logistics and supply chain and just the *Lean* ideas can make a contribution in this domain.

How to implement the *Lean* concept in agribusiness sector? The key thing is to define a vision - what the enterprise wants to achieve? What must not be an empty phrase, but something that can be measured? It is important that employees and management believe in the vision and contribute to its achievement. When the vision has been defined, it is necessary to choose adequate instruments of the *Lean* concept that are appropriate to the vision and specific characteristics of the enterprise.

One possibility is the use of so-called *t-card*, which ensures that assignments should be marked visibly, both those that were made and ones that should be done. The above-mentioned method was successfully applied on farms in Sweden, while the operationalization of this technique has been performed by using billboard and posting on a daily basis all assignments according to the priority of their implementation. In addition, each individual obligation or task was highlighted on individual cards. On the red side of the card there are listed the tasks, and after their implementation the employees are turning back of the card that is green, which signals that the task has been completed and that they can keep on with fulfillment of other obligations. The assignments are displayed in several rows (the morning duties, obligations that need to be completed during the day, the evening duties), and when all duties have been completed, only green cards can be seen on the billboard (Pejstrup, 2015).

The application of *Lean* principles in agriculture also includes an ethical dimension which is reflected in the following: respecting of employees, respecting of animals, respecting of enterprise and respecting the environment. This means that the path to success (higher productivity and result) in addition to optimal management requires not only adequate accommodation and treatment for animals, but also the learning and development of employees at all levels in an enterprise. Only through raising awareness of employees it is possible to achieve goals together with preserving the environment and respecting of ethical principles. .

The implications of Lean systems on economic and environmental performances

In order to be adequately perceived the potential of the *Lean* concept of production onto the enterprise performances and their improvement, first of all it is necessary to consider the conditions which are typical for the traditional understanding of the production and the side effects that follow this concept. Accordingly, the comparative overview of the characteristics between the traditional system and the *Lean* concept of production are given in Table 1 together with the more detailed textual explanations.

Table 1. Comparison of the traditional and the *Lean* production concepts

Traditional production concept	Lean production concept
Implementation of the plan	Orientation on goals
Mass production	Serial production
Overproduction and economies of scale	Focus on customers
Surplus of inventories	Just in time
The existence of significant write-offs	Total Quality Management
The lack of synergy	Teamwork and synergy effect
Physical work	Creativity
Standardization	Innovations
Maximization of profit	Optimization of economic and environmental performances

Source: George, 2010.

Traditional system of production requires a significant consumption of resources, since it is producing for storage and not for customers, which significantly exhausts and degrades natural resources. In fact, this has resulted not only in significantly increase of production costs, but also the total expense, causing the economic results that could be less

than the optimal ones. Although it can be expected that with the increase in production volume the unit cost would be reduced, the accompanying costs incurred due to the occurrence of write-offs (loss of resources, costs of the waste handling, fines and penalties regarding the pollution) significantly annul digression of the unit cost and moreover convert themselves into the negative contribution to the overall business results. In addition, a higher production volume has been followed by the larger volume of defective products, which requires some more finishing and spending additional resources. In the area of stock management, a greater volume of stocks requires the use of a greater packaging material quantities, a higher risk the product expiration date, more storage space, additional costs of insurance, which all increase the total stock costs. Transport and manipulation of a larger volume of products toward warehouses means higher energy consumption, the potential damages and pollution (Moreira et al., 2010).

It should be noted that the case of excessive production (a higher production than the market or customers' needs) is followed by the higher use of resources, equipment, labor force and space, which further exhaust the existing natural resources and contribute to a high level of environmental pollution.

Contrary to the previously highlighted limitations of traditional production systems, the *Lean* production concept is aimed at the realization of goals that are primarily related to the improvement of product quality, reduction of the waste, an increase of the customers' and employees' satisfaction, innovation, creativity, environmental protection, contribution to the sustainable development and optimization of the economic and environmental performances.

According to the traditional concept, an increase of the business results increase primarily derives from an increase of the production volume, but the rise in prices of products and services as well. In other words, there is an attempt the costs to be passed on to customers. However, the globalization of markets and growing competition have caused a need for an improvement of the performances through the reduction of costs for a given level of prices, i.e. the performance to be improved by the efforts of employees through the continuous improvement of the business processes and simultaneous elimination of all irrationalities and associated inefficiencies in spending of resources.

An application of the *Lean* concept contributes to the reduction of direct labor costs, and then it reduces indirect costs and time of product delivery, with simultaneous increase of the customer satisfaction level and return on investments. More precise results achieved in practice by switching from a traditional production concept to the *Lean* business concepts are given in Table 2.

Table 2. Improving of performances in the *Lean* production conditions

Operating margin	Improved from 5.4% to 13.8%
Capital turnover	Improved from 2.8% to 3.7%
Return on investment capital	Improved from 10% to 33%
Market capitalization	Improved by 225%
EBITDA	Improved by 300%
Economic profit	Improved from -2% to +21%

Source: George, 2010.

Previous results clearly confirm the potential of the *Lean* production concept and importance of the change in way of thinking through the abandonment of the traditional

production concept. In addition to the economic performances, the *Lean* concept that insists on the so-called clean production provides a contribution to the protection and preservation of the environment. In the area of environmental performances, which has been increasingly monitored in the recent years, especially in the areas where there are significant polluters e.g. the enterprises in the manufacturing industry and agribusiness, the emergence of waste exacerbates the ecological performances, contributes to emission of harmful gasses and jeopardizes the enterprise business in the long run. Through more efficient use of resources i.e. higher productivity of resources (greater effects achieved per unit of used resources) on which the *Lean* concept particularly insists, it has been reduced the intensity of materials and energy utilization, which at the same time has increased the enterprise eco-efficiency. Previously presented attitudes support the fact that *Lean* concept offers significant potential not only to improve performances in the short term, but also to contribute these results take on a permanent character.

Conclusions

The *Lean* concept is an instrument for improving the business operations in the contemporary environment. This is one of the most potent methods for improving the enterprise productivity. The basic principles of *Lean* concept are the following: focus on the customers, creating of the added value, orientation to the goals, innovativeness, and the waste reduction. As such, this concept can represent a significant factor in improving of innovativeness, quality, competitiveness and overall performances of the agribusiness enterprises.

The *Lean* concept should contribute to the reduction of business complexity through focus on the idea itself, on thinking, finding out alternative ways for job implementation in a more efficient manner, with the reduction of waste. Since the agricultural sector emerges as a significant factor in environmental degradation, reduction of waste at the same time contributes to an improvement of the environmental business performances, i.e. to the "cleaner" production. Reduction of ecological costs further results in an improvement of economic performances, primarily in the form of higher profitability and cash flow generating, which is an important assumption for innovation and higher efficiency in the future.

It should be particularly emphasized that the *Lean* concept contributes to an increase of the enterprise flexibility through innovative solutions of the problems, i.e. it strengthens the ability for timely reacting to changes in the environment, which is of crucial importance for the sustainable success of enterprises in the conditions of the growing business operation challenges. This characteristic is particularly important for the agribusiness sector whereas, due to numerous external constraints, any delay or inadequate response could have multiple repercussions on the future business performances. The aim should be to organize the business operations in a way that every aspect of business should function efficiently, without delays and unnecessary wastage of resources, and also that the human, material and biological resources should contribute to the implementation of the *Lean* goal.

Starting from the fact that agricultural production is a significant source of pollution, as well as the previously described effects of the *Lean* concept in terms of reducing waste and pollution, the use of smaller amounts of resources to achieve the same or higher results

and future research in the field of application of the *Lean* concepts in agribusiness, could move in the direction of his contributions and achievements in solving the problem of excessive depletion of natural resources, an imbalance of ecosystems and mitigation of the global warming problem. Namely, all of the cited risks could jeopardize not only the possibility of a sustainable business operation of agribusiness enterprises, but also maintenance of the existing level of the life quality on our planet.

Note

The paper is a result of the researching project No III-46006 «Sustainable agriculture and rural development in the function of implementing the strategic goals of the Republic of Serbia within Danube region» and project No III 46001 “Development and application of new technologies in production of competitive food value-added products for domestic and foreign markets – Let’s make wealth from the wealth of Serbia” financed by The Ministry of Education and Science of the Republic of Serbia.

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UDC: 338.436(497.11)
Original scientific paper

MODELS OF NETWORKING IN AGRICULTURE OF SERBIA⁶

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Abstract

Business networking has become a modern strategic need and the global trend. Through the cooperation of economic actors in the agrarian sector of Serbia enhances their market position and bargaining power in the "value chain". In addition, strategic management network enables organizations to decrease costs, increase profits and better responsiveness to changing market conjuncture. The rich knowledge in the business, increasing the importance of concentration researchers, consultants and laboratories in one location, and thus increases the possibility to create such innovations, ie the value and quality of the offer, that distant rivals can not easily be copied and overcome. The paper analyzes three models of networking in the agrarian sector of Serbia: clusters, cooperatives and business incubators. Practice has shown that networking organizational structure, business processes, and scientific research etc. comes to the key knowledge, skills and other benefits that are on the market valorized as competitive. The greatest chance of success are those associations in agriculture that have consensus on common objectives and activities, which have a clear framework for cooperation and which are created based on your own initiative.

Keywords: networking, clusters, cooperatives, business incubators, competitiveness.

Introduction

In developed economies of the world there are nowadays different forms of association in agriculture. Consumers cooperatives are the most developed in Great Britain, producers-workers' cooperatives in France, credit unions in Germany and agricultural cooperatives in France, Germany, Denmark, Italy and the Netherlands. In those countries have also been considerably represented service cooperatives (housing and health care cooperatives). The agricultural cooperatives are the most important in countries in which dominate farmers and peasants husbandries. In agricultural countries of market economy with development of productive forces are revealed the concentration of capital in activities which produce means of production for agricultural needs and deal with finishing, processing and turnover of agricultural products as agribusiness segments. Those processes at the same time cause the changes in primary production. There comes to intensification of production, the

¹ Paper is a part of research within the project no. III 46006 - Sustainable agriculture and rural development in the function of accomplishing strategic objectives of the Republic of Serbia in the Danube region, financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

production structure changes, the subjects from the primary production connect with capacities of processing industries and trade organizations.

A key goal of business networking consists of realization of their useful economic and organizational effects (direct and indirect). It explains in literature through its fundamental principles of establishment and a functional specificity of existence, which reflects in a dynamic tendency of organizational development, constant expansion and specialization aiming to achieve as better position and success on the market as possible. In that sense business networking substantially represents a self-organized polycentric structure, oriented to the concrete tasks.⁷

Network economy significantly changes performances of international trade and competition in general. The business networking doesn't appear as a substitute of traditional hierarchical-bureaucratic organizational-managerial structures, but as a new management strategy, even a paradigm used by many world (especially global) companies which base their success and development on a modern structuring of business processes. The networking improves the abilities of adjustment to changes, innovativeness, modernization and training in regard to the hierarchic-bureaucratic organizational structures.

Development, nature and characteristics of cooperatives in Serbia

The international cooperative alliance had persistently worked in its one-century long existence as a guardian of cooperative values. On the other hand, in many countries' practice, the cooperative movement had been encumbered by different dilemmas, ambiguities and confusion carried by constant changes in the world we live in.⁸ For revival of the cooperative movement through the transition process for our countries can be very useful historical and modern experiences of countries with developed market economy, which cooperative organizations use as an alternative to the modern capitalism in several directions. Among those directions are the principles of:

- Preservation of human dignity through self-employment,
- Equitable distribution of created national wealth,
- Economic and social emancipation,
- Greater individual responsibility toward the community he lives and works in,
- Raising general awareness for sustainable development, etc.

In the field of cooperative identity innovation there are neglected institutional researches. The public has not been well informed on this problem. In order to perceive a real value of the cooperative movement, which was built on the existing ideas and concepts, it is necessary to spread knowledge about the movement. Thereby it is necessary to accept the prevalent concept present in every form of cooperative, which reflects in the following: group of people, small or large, access with loyalty to a joint action based on democratic principles and with mutual support in order to provide a business activity or services useful for all participants of these actions.

² Drašković, M. (2006): "Klasterizacija kao komponenta globalne konkurencije", *Montenegrin Journal of Economics* No 4, str. 168.

³ Simonović, Z., Arsenijević, Ž., Mihailović, B. (2008): "Zadrugarstvo Srbije u uslovima evolucije i tranzicije", *Economics of Agriculture*, No. 3 (237-338) 2008, pp. 319-328.

In our further presentation we have paid our attention to the problem of the Serbian cooperative evolution, which comprised the period from 1957 to 1990, but also the transition, which has started since 1990 when the Serbian cooperative had entered a new phase of its development. Transition lasts to the present day. The Serbian cooperatives had undergone many trials in past fifty years. This was the period which had encircled time of socialism, but also the modern period characterized by the transition. In each these movements, i.e. different experiments which had happened to be, the Serbian cooperative still had managed to survive. This shows how institution of the cooperative is resistant and capable to survive in all social-economic systems. Modern Serbian cooperative has caught up with application of newest cooperative principles, which was also valid internationally, according to the draft of the Law on Cooperatives. Agricultural cooperatives in our region have not been mostly powerful organizations in economic sense, but they are necessary for small and medium-sized agricultural producers, which in most of cases could neither base their production, nor to make concrete terms for realization and payment for their products. At the same time, the cooperatives have a great significance for processors, intermediaries in trade and final consumers, which buy via cooperatives the adequate quantity of goods, in one place, instead of negotiating and buying with numerous agricultural producers.

Modern cooperative sector of business activity bases on founded cooperative principles and values. It was built on businesses which contributed to economic and social welfare of wide range of people of different professional orientation.⁹ Developed cooperative sector contributes to its members' benefit through provision of new services and development of new businesses on cooperative basis. Lack of clear visions and strategies of association in agriculture sector has caused also the agrarian policy which represents the heritage and prolongation of policy from the socialist period, without significant structural changes. An important part of agricultural land is in private ownership, which provides major part of an agricultural product. Also, the policy which implements is oriented to provide assistance and to maintain the agricultural land, which was formerly socially owned, along with gradual guidance of the policy toward the commercially oriented agricultural producers. Consequentially, the agricultural sector in Serbia is not capable to engage itself in an equal match with competition from the EU countries. It is extremely difficult to compete with the surrounding countries due to accumulated problems which refer to:¹⁰

- commodity exchange for inputs, mostly agricultural products, covers up the real costs and values of products, so that agricultural producers do not get real signals on prices, but their potential profit overflows to others,

- Land market, which should provide adjustments regarding the size of land, as well as more efficient structure of households, doesn't work properly. The reasons lie in an obsolete register of land and a cadastral system, impossibility to offer for sale or lease the land of the state agricultural husbandries, lack of long-term crediting for land purchase, unsuccessful apply of law for paying the lease, as well as the uncertainty in wider economic sense which discourages the long-term investments,

- the market of rural credits hardly operates due to the general lack of crediting, lack of liquidity, knowledge and experience in banking sector for agricultural credits, absence of

⁴ Zakić-Vujatović, Z. (2000): *Coop management*, Dunav Grupa, Dunav Preving, a.d., p 17.

⁵ *Strategy of agriculture of Serbia*, Ministry of Agriculture, Forestry and Water Management, Belgrade, 2004, pp.8-9

agricultural profitability, high risk, absence of rights over land which could provide guarantee,

- Privatization is slow, with poor foreign investments, so the state still represents a main carrier of ownership in many processing factories and major of combines (factory farms). In other transitional economies foreign investments are a main form of industry revival, as in Serbia is not the case,

- Market infrastructure, institutions and organizations develop slowly. Efficiency of production is low and there is also lack of knowledge on market performance and marketing. Production performance in many husbandries is very low. Processing capacities, unlike producers, do not have problems with small capacities and fragmented production, but often the monopoly and high level of subsidies through cheap credits and debts write-off, seem to maintain the inefficient production,

- Working of market is limited by deficient legislation and absence of an institutional frame, which especially hinders the engagement of commercial family husbandries in distribution of agro-food products,

- Institutions which support have not been adequately qualified in technical, organizational and personnel sense.

It is necessary to support joining and cooperation between agricultural producers, especially a horizontal one (for example, public grants which support making new cooperatives or production associations, or trainings and seminars aiming to strength cooperation culture). The horizontal cooperation in agricultural production represents a vital need, because the individual producers have no strength to negotiate effectively on prices and other terms with big distributive companies, as well as the strength to invest in innovations and quality of products, in order to increase their own competitiveness. By joining the agricultural producers achieves a synergy in business and reaching critical mass in improvement of the negotiating position, which affects improvement of the agrarian competitiveness.

Structure and factors of clusters development

According to Porter, strong competitive advantages in the global economy lie mostly in local issues – knowledge, relations, motivation – differences which competitors could not copy easily, and which can be develop through clusters.¹¹ The cluster association is characterized by cooperation and connectivity (unification and complementarity) of members, their geographic, i.e. local boundedness, active channels for business transactions and communications, making a common product and/or a service or joint solution of some need or a goal. Main factors in clusters development can be companies-members. Only through their active participation, a cluster will grow stronger and develop. Educational institutions also have their role and they showed in some cases as an important catalyst in clusters development.

Universities may have educational role, but they also can be the key factors in research and development, as well as innovation in the clusters. An integral part of clusters are the organizations for providing business services with an expertise which is adequate to

⁶ Porter, M. (1998): *Clusters and the New Economics of Competition*, Harvard Business Review, November-December, p. 78.

clusters needs, like marketing, consulting and other organizations. All these bodies can contribute to clusters development strengthening, and they can have a legitimate role in its development. Very important characteristic of clusters, which results from its essence is knowledge. In most of cases, clustering by the “bottom up” line leads to so called quasi-clusters, to be more accurate-the associations, which pretend to become clusters. The following “clusters”, i.e. the associations are worth mentioning: association Fruitland, cluster of agricultural producers in Kraljevo, “Rakovica agriculture cluster”, association of vegetable producers Begec.¹² These „clusters“were initiated by members not for the reason to obtain state incentives but to make a better position of the companies within the cluster on the market.

Analysis of the existing cluster initiatives in Serbian agriculture shows that the local authorities, regional developmental agencies and other corporate entities have a significant share in encouraging the development of clusters by interventions, strategic directions, donations, making favourable conditions for development, organizing educational seminars by the regions, decreasing the risk for starting a business or while taking credit, etc. Accordingly, it is noticeable that there are several common elements in the way a cluster works:

- Core businesses – businesses as leading participants of clusters and most often bring the highest incomes from users outside clusters.

- Supporting businesses – businesses which directly and indirectly support basic business of clusters. Here belong the suppliers of specialized machines, components, raw materials, as well as other service firms, including also the financial/broker firms and their capital, lawyers, designers, marketing services and public relations services. Those firms are mostly highly specialized and they are located in the vicinity of core business firms.

- Intellectual supporting infrastructure – in clusters which operate top-class, the core and supporting businesses do not operate separately, isolated. In successful clusters many members of a local community participates: local schools, universities, polytechnic institute, local trade associations, centres for economic development and many others which support the activities of a cluster and they are key factors in clusters. Quality of this intellectual supporting infrastructure, as well as the level of team work within the infrastructure, is very important factor in development of any cluster.

- Physical supporting infrastructure is, first of all, physical infrastructure: roads, ports, settled dumps for a classic or specific waste, communication network, etc. Quality of such infrastructure must be at least at the same level as the competitive one, and it is preferable to be even much better.

Contractual communities of agricultural producers in Serbia represent a valuable base for establishment of clusters. There should help agricultural producers, orally and in writing, and also with the best realized project to decide for production association, to choose a form of association. Realized results in implementation of certain solutions are the best groundwork both for the selection of form and way of production association.

⁷ Mihailović, B., Paraušić, V., Simonović, Z. (2007): *Analiza faktora poslovnog ambijenta Srbije u završnoj fazi ekonomske tranzicije*, Institute of Agricultural Economics, Belgrade, monograph, pp. 120-122.

Certain forms of association that have wider application in some developing countries can be implemented in our conditions.

Contractual community of arable farmers.

It usually mixes several agricultural producers (4-5) within or outside a cooperative, so they could rationally use agricultural mechanization in their husbandries. However, these contractual communities in Serbia should be larger according to a number of arable farmers, if we are to take on the exploitation of powerful tractors and their auxiliary machines, and in the present size of our family farms. Besides, when joining larger husbandries, there more powerful tractors within each individual husbandry can be used, because they manage with parcels of the appropriate size (5-10 ha). Taking it into consideration, we should seek for the adequate solutions by way of a special performance of land consolidation. Except grouping the land by ownership, the land consolidation should be characterized also by grouping the land of the same type of exploitation. The associations of arable farmers would be followed by joining parcels. The costs of production would decrease and performing mechanical activities would be easier by joining arable farmers, because the machines are fully exploited on a larger surface of land of associated farmers.

1. Contractual community of arable farmers, vine growers and users of other plantations. Such communities often form cooperative farmers or producers outside the cooperative, who have their parcels in certain preferred positions, and they want to take advantage of a large-scale (plantation) production.¹³ Plantations raise in the land of agricultural producers as a unique territory, independently on boundaries of individual agricultural producers' parcels, which can be marked, in order to mark the ownership of an individual. All activities, both in raising and utilizing that can be performed mechanized, perform in plantation independently on some parcels boundaries. Every manual work performs an individual in his parcel, or better to say – his rows, which have been assigned to him proportionally to his land. In distribution of jointly produced product, they participate according to invested work. Transition to modern organized production frees cooperative members of hard manual works, and at the same time it provides sale of raw materials.

2. Contractual community of livestock farmers. Agricultural producers can associate in order to produce milk jointly. A herd is of size which provides rational business. These contractual communities of milk producers have significant advantages in regard to pretensions in our country to form mini-farms in terms of small family husbandries. Instead of 3 stables (each for about ten cows), it is cheaper to build a stable for 28-30 cows. Instead of three rooms with devices for cooling milk, it can build one with better mechanized devices. In our case, each producer must do some activities in the stable, which turn away many people to devote to this job. It is easy to understand the advantage of contractual communities in which are engaged the members of contractual community, without adduction of other rationalities in stables of larger capacities. It is natural that livestock farmers' communities can form in other lines of livestock production, too. Clusterization of Serbian economy is at the very beginning and clusters contribute to

⁸ Mirić, S. (1994): *Zadružno-proizvodno udruživanje poljoprivrednika*, A century of Serbian agricultural cooperatives, Belgrade Cooperative Union, Belgrade

development of competitiveness, through the productivity growth and creation of innovative strategies, which cannot be lost out of sight in the following period, when there should expect more intensive processes of clustering.

Business incubators as an assumption of Serbian agrarian sector development

Business incubators are the instruments of a local economic development for support to newly opened small enterprises in the first years of their growth and development, when they are the most vulnerable.¹⁴ In order to minimize ignorance and inexperience in management, accountancy, expertness in market and running a business in the initial phase of these new small enterprises in agrarian sector of Serbia, these roles have been put together through the common services and occasionally guest performance of the consultants who have done these jobs for all enterprises in a hall – incubator of new enterprises. According to the same source, the most important services, which provide the capacities for incubation, enclose an organized access to expertise, experiences, consulting services, advices and mentoring in key business networks and groups.

Offer of physical facilities and these services to entrepreneurs increases the probability for success of an enterprise in regarding to some other which does business independently: research at the EU level show that mortality rate (in the first five years of business) of enterprises, which have started their business in the business incubator, was less than 15%, unlike those other enterprises with the mortality rate of 50%. The incubator works as a capacity in which enterprises go in and out, i.e. their stay in the incubator is limited (usually about 3 years).

Consulting services of business incubators to members, but also to wider local community, create in regard to the expressed needs, world practice in work of business incubators, but also local peculiarities. They are significantly determined by the available staff, finances and technical equipment. The consulting services provided by the business incubator are of the vital significance for development of enterprises-members, but also the business incubator. They can also be the source for providing additional funds in order to decrease dependence of the business incubator from subsidizing and donations and they can be crucial for self-sustainability.¹⁵

All consulting services provided by the business incubator are susceptible to changes, so some new can always add or some old expelled, depending on needs of the local community and members of the incubator. At the same time, a price of the consulting services can be changed depending on inflation, costs and supply/demand, but it should always be more favourable for members than market conditions and at the same time structured in a way to ensure income sufficient for the incubator sustainability. An adequate ratio between the consulting services and their price makes the business incubator into a very efficient mean of the local economic development.

Most of the consulting services should be included into a price of the membership fee for enterprises-members. According to the report of the European Commission

⁹ Danilović Grković, G., Kovačević B., Sedmak. A., Nedeljković. M. (2005): “Uspostavljanje biznis inkubatora tehničkih fakulteta u Beogradu”, *XIII Telekomunikacioni forum TELFOR 2005*, Beograd, Sava Centar, 22.-24.11.2005.

¹⁰ Support to foundation of business incubators in southern Serbia, Feasibility study of the project “Business incubator in Vranje”, January 2006; Analysis made by the expert team of Timok Club; Team leader: Dragan Milutinović.

“Evaluation of business incubators” (2002), “The basic value of business incubators work lies in a type and quality of business support to the clients. There are four key fields: training of entrepreneurs, business consulting, financial support and technological support.”

The goal of business incubators has to be setting up the structure and form which suit the most to the specific needs of the local and regional economy. However, they cannot make economic development by their own. The business incubators, whether they are within the scientific-technological parks or outside them, must be a part of a broader strategy or program of economic development which refers to every dimension of economic changes, especially those in transition, where the incubators have shown the most efficient within the broader programs for development of small-sized enterprises sector.

Other forms of networking in agrarian sector

Taking into consideration that properties in Serbia are fragmentized, as well as dispersion of necessary equipment and mechanization, by creating the “machinery rings” would realize more efficient production due to more rational use of the existing resources, whereby there would fill up simultaneously the economy of scale and the economy of size. The machinery rings are a special form of agricultural producers' organization in countries with developed agriculture (prevalently in West Europe and Japan). A basic idea is to use an agricultural machine in the most rational and the most productive way. With this approach to the problem of land cultivation has come to division of agricultural producers into two basic categories, those who receive and those who provide services. This division has led us to services providers, who are narrowly specialized only for the specific operations, so the quality of provided services is at the highest level. Besides, by the maximal utilization of mechanization, a price of such provided services is lower. On the other hand, receivers of services can commit themselves to other problems in their agricultural production (inputs, sale of products), and not be handicapped by their “unutilized” mechanization.

In such conditions, the cooperatives should be oriented in their future work to businesses linked to the cooperative members. It is necessary to base their activities in the future period on respect for the cooperative principles. The cooperatives must restructure in organizational way, they also have to expand membership, formalize mutual relations by adequate normative acts and ensure their persistent implementation. It is necessary that cooperatives and cooperative unions, along with support of the state authorities, enable the cooperative audit in order to sanction successfully former and prevent future negative manifestations e.g. that only employed workers can be members of the cooperative and that only them can manage with the cooperative; not to enforce the law on cooperatives, etc. Thereby the cooperative would become the organizations of cooperative members in essence.

Conclusions

Important assumptions for increase of agricultural production of Serbia reflect in the following goals which should be achieved. These are production restructuring, ownership and management adjustment to a new situation. All of these should make better business

results in agricultural production. On the other hand, disintegration of social agricultural cooperatives and combines has left a free space in organizing agricultural producers in many regions. They have been left alone. There can be generally concluded that there are not enough agricultural cooperatives in Serbia for the reason of lack of knowledge of agricultural producers on the original values of the system of cooperative societies. Cooperative practice of the socialist period had significantly discredited an idea of cooperative self-organization. This period characteristic is that cooperatives had governed over agricultural producers and not the opposite.

Modern business conditions of agricultural producers contribute in many ways to their strategic thinking, which has been mainly directed to as efficient cooperative movement as possible. Association of agricultural producers makes easier their joint performance on increasingly demanding market.

The association can observe through many dimensions. Pooling resources and financial strengthening are the two most important components for long-term cooperation of cooperative associations. A certain kind of discipline suggested to them will not be easily achievable. Besides these two elements of strategic thinking there are also other elements which imply safety preservation of the cooperative movement generally, along with cooperation with the government institutions.

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THE PRODUCTION AND USE OF BIODIESEL AS AN ENVIRONMENTAL FUEL IN THE FUNCTION OF SUSTAINABLE AGRIBUSINESS

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Abstract

Fuels produced from biomass are one of the most promising alternative and renewable fuels. Biodiesel is a methyl ester produced from vegetable or animal fats diesel quality. Trends of biodiesel production have been intensively rising. From 2000, biodiesel production has been increased for over 3 times. Bearing in mind the multiple benefits of biodiesel production, this paper analyzes the application of biodiesel in the modern business environment. The authors emphasize the role of biodiesel for environmental protection and for sustainable agribusiness achievement. The practical objective of this paper is to draw attention to the environmental, economic, technical and technological aspects of the use of biodiesel in agribusiness. Serbia has significant potential of the land for the production of oilseeds for processing into biodiesel. Oilseeds are grown in Serbia on about 270,000 ha, but it is estimated that Serbia has the real potential for an area of over 620,000 hectares. These facts suggest that potentials of the production and use of biodiesel in the Serbia are not adequately recognized and valorized. Therefore, one of the objectives of this paper is to highlight the importance of increasing production and use of biodiesel in the Republic of Serbia. The analysis of the role and importance of production and use of biodiesel in the function of the sustainable agribusiness is based on contemporary national and international theoretical knowledge and relevant sources and applies adequate qualitative methodologies. The methodologies used are: method of analysis and synthesis, comparative method, inductive and deductive method.

Key words: biodiesel, oilseeds, environment protection, sustainable development, agribusiness.

Introduction

Scientific-technological development and uncontrolled use of natural resources during the 20th century negatively affected environment and climate changes which are gradually endangering people's lives. Damaging of biosphere and exhaustion of many natural resources, pollution and degradation of air, water and land, endangering of health and

descending quality of life – are the basic manifestation forms of ecological crisis which affected the Planet at the end of the previous century. Due to these specific problems, numerous researches have been conducted for the last several years in order to discover renewable, alternative energy sources and to decrease the use of non-renewable sources and their negative influence on environment and people's health. Conducted researches showed that the most perspective, renewable fuels are the ones obtained from biomass. Biodiesel, obtained in the process of transesterification of oils or fats, is especially distinguished in the group of alternative ecological fuels.

Materials and methods

Biodiesel is a methyl ester produced from vegetable or animal fats of diesel quality. Use of this biodegradable, non-toxic fuel produced from renewable sources is characteristic for low emission of pollutants. Due to the data on reduced pollution of environment in biodiesel use and the fact of being produced from renewable resources, nowadays biodiesel is a highly attractive energy source. Biodiesel is used in pure form (B100) and as a mixture with fossil fuel in different concentrations (B5, B10, B20), when biodiesel improves lubricity, filterability and cetane number of fuel (Ma and Hanna, 1999; Fukuda et.al., 2001) Biodiesel specifications are presented in the Table 1.

Table 1. Biodiesel specifications

Biofuel characteristics	Units	Lower limit	Upper limit	Method of testing
Esters	% (mas.)	96,5	-	pr EN 14103d
Density (15°C)	kg/m ³	860	900	EN ISO 3675/ EN ISO 12185
Viscosity (40°C)	mm ³ /s	3,5	5,0	EN ISO 3104
Flash point	°C	> 101	-	ISO CD 36796
Sulphates	mg/kg	-	10	-
Carbon residue (tar, 10% distillation residue)	% (mas.)	-	0,3	EN ISO 10370
Octane number	-	51,0	-	EN ISO 5165
Sulphated ash	% (mas.)	-	0,02	ISO3987
Water	mg/kg	-	500	EN ISO 12937
Total contamination	mg/kg	-	24	EN 12662
Cu corrosion (3h/50°C)	classification	class 1	class 2	EN ISO 2160
Thermic stabilitv	-	-	-	-
Oxidation stability (110°C)	sati	6	-	EN 14112
pH	mg KOH/g	-	0,5	pr EN 14104
Iodine	-	-	120	pr EN 14111
Linolenic acid ME	% (mas.)	-	12	pr EN 14103d
Polyunsaturated (≥ double bonds) ME	% (mas.)	-	1	pr EN 14103
Methanol	% (mas.)	-	0,2	pr EN 14110l
Monoglyceride	% (mas.)	-	0,8	pr EN 14105m
Diglyceride	% (mas.)	-	0,2	pr EN 14105m
Triglyceride	% (mas.)	-	0,2	pr EN 14105m
Free glycerol	% (mas.)	-	0,02	pr EN 14105m/ pr EN 14106
Total glycerol	% (mas.)	-	0,25	pr EN 14105m
Alkali metals (Na+K)	mg/kg	-	5	pr EN 14108/ pr EN 14109
Phosphorous	mg/kg	-	10	pr EN 14107p

Source: Jovanović, S., Jovanović, L. (2009). Perspectives of biodiesel use, *Ecologica* 16/2009, no 54, 123-127, p. 124, Belgrade.

Increased interest in biodiesel was noticed during last decades. EU countries, the USA, Thailand imposed intensified tempo in biodiesel use as a substitution for fossil diesel. Europe produces 95% of biodiesel mostly from rapeseed and sunflower seed. In 2003 EU defined obligations for all EU members to substitute fossil diesel with biodiesel, deadlines with substitution percentage, control and monitoring of implementation as well as financial “penalties” for the members who resist defining substitution amount. There were predictions that biodiesel market could take 10% of diesel market in Europe until 2015. The USA suddenly increased biodiesel production which was 1, 9 million litres in 1999 to 95 million litres in 2004. American government agency estimates that biodiesel and ethanol could substitute between 25% and 50% American fuels obtained from oil until 2030. These assumptions match expectations that total energy should be provided from renewable resources until 2100. Other countries followed the example as well. In February 2005 China passed the law that 10% of energy would be obtained from renewable resources until 2020. India could be an important biodiesel supplier for its potential consideration that the price of biodiesel processing is a third of the price in Europe (Premović, T., Milićević, Z., 2015).

Results and discussion

Biodiesel effects on agricultural production – sustainability of agriculture

Principal advantage of biodiesel use as a renewable fuel is a considerate reduction of CO₂ emission. Emission of sulphur oxides, suspended particles and carbon monoxide is reduced as well. Advantages and deficiencies of biodiesel use depend mostly on the type of used mixture as well as on performance and type of engine. Importance of potential reduction of certain pollutant emission during biodiesel use is in the following (Košut et.al. 2008).

Carbon dioxide (CO₂): On WTW basis, each ton of fossil diesel adds about 2, 8 t of CO₂ in the atmosphere. The specific content of carbon in one ton of biodiesel is a bit lower, 2, 4 t of CO₂. It is assumed that this carbon will be totally used by the crop giving the raw material for vegetable oil production as well as absorbed through carbon cycle (as glycerol and solid waste). In this way the “greenhouse effect” is alleviated as the current problem on the level of the entire atmosphere. Thus, it is said that the net CO₂ emission in biodiesel use, observed on WTW basis, is almost equal to zero (Fukuda et.al. 2001; Furman et.al. 2006; Košut et.al. 2008).

Sulphur oxides (SO_x): Nowadays 1 t of conventional fossil diesel in EU contains maximum 350 ppm of sulphur on average. When diesel is burned, sulphur is released into the atmosphere in the form of sulphur dioxide contributing to formation of acid rains. Biodiesel contains almost no sulphur (sulphur content is 0-0, 0024 ppm). On the other hand, EU constantly promotes the use of diesel fuel with low sulphur content below 50 ppm (Great Britain), below 10 ppm (Sweden) (Košut et.al. 2008).

Nitrogen oxides (NO_x): Emission of nitrogen oxides from biodiesel can be increased or reduced compared to the emission from fossil diesel depending on the engine generation and testing procedure. Nitrogen oxide emission from pure biodiesel is increased for about 8% on average compared to fossil diesel (Košut et.al. 2008). When the mixture of small biodiesel amount and fossil fuel is used, the emission of greater quantity of nitrate oxides is possible which could be eliminated by adding additives for better combustion (Furman et.al. 2006). Considering the lack of sulphur in biodiesel, it is possible to apply control

techniques on nitrate oxides which are impossible to apply on fossil diesel (Košut et.al. 2008).

Carbon monoxide (CO): Biodiesel contains oxygenates improving the process of combustion and reducing emission. This fact considerably reduces (at least 20%) carbon monoxide emission.

Solid particles: Inhaling of suspended particles is proved as a serious problem and danger for people's health. Emission in exhaust gases of these particles is 40% lower in biodiesel than in fossil diesel (Košut et.al. 2008).

Increased use of biodiesel from the local areas improves micro economy of urban and rural regions. According to the data of the US Department for Agriculture, food price was increased for 5, 1% in 2008 whereas 0,2-0,6% goes to the biodiesel influence (biodiesel, bioethanol). Greatest effect on food price increase is the fuel price increase: in America food is transported to great distances mostly by trucks so the fuel price directly influences food price.

Primary reasons for food price increase are:

1. price of oil included in the price of fertilizer and transport
2. increased consumption, population growth and improvement of nutrition quality in developing countries
3. climate conditions resulting in poor crop, floods, etc.
4. export restrictions of certain countries.

Despite high fuel price, technological development leads to reduction of food price. In this way, increase of fuel price due to the increase of food price is not expected (Jovanović, S., Jovanović, L. (2009). Biodiesel has the highest energy balance compared to other fuels used in transport industry. New analyses show that the energy balance of biodiesel is positive (3,5:1). In other words, each unit of fossil fuel used to produce biodiesel results in biodiesel quantity containing 3,5 energy units. This study was conducted at the University of Idaho in cooperation with the US Department for Agriculture. The calculation excluded fuel consumption for planting, harvesting, fuel production and transport to the user.

Economic aspect of biodiesel use – economic sustainability of biodiesel

Biodiesel produced from vegetable oils and animal fats is more expensive than diesel obtained from raw oil for 10-50% (Leung and Guo, 2006). High price of biodiesel is the main obstacle for its commercialization (Predojević and Bošković, 2009). It is known that the price of raw material is 60-75 about 80% of biodiesel total price (Thurmond, 2008), however, in the economic analysis of biodiesel production it is necessary to observe all the parameters influencing the price forming of biodiesel in the production chain consisting of oilseed production, their processing into raw oil and biodiesel production (Ma and Hanna, 1999; Jovanović and Zekić, 2007).

Basic condition for efficient biodiesel production and use is an adequate selection of raw material. Considering the content of oil in seeds and the price, the most optimal raw material for biodiesel in our region is rapeseed. However, current areas of this crop cannot be the raw material base for significant biodiesel production. Therefore, according to the previous research, one of the solutions would be the increase of the purchase price or implementation of various state incentives (Kiš et.al. 2007).

Researching economic characteristics of biodiesel production, Kiš et.al. (2007) also concluded that biodiesel production from rapeseed was economically optimal. These authors published results of the conducted analyses on biodiesel production total costs with 59,6% of costs on the rapeseed raw material. Therefore, the authors emphasized that special attention should be dedicated to the analysis of current and prediction of the future development on the raw material market. Results of the conducted research determined the price of biodiesel as 41,6 dinars/l and that at the regular 18% tax rate is still lower than the selling price of D2 fuel, providing conditions for the profit in production.

Jovanović and Zekić (2007) also analysed economic properties of biodiesel production from rapeseed. These authors determined material costs of oilseeds processing into biodiesel fuel in accordance with value of raw material (rapeseed) calculated from the market price, raw material processing costs (cleaning, crushing and pressing of the seed) and costs of raw oil processing into biodiesel fuel (methanol, sodium, hydroxide, sulphuric acid, distilled water, heat and electric energy production). Costs of fixed assets were determined as the sum of costs for amortization, maintenance, insurance and interest on equipment and buildings. Labour costs for direct workers and workers in general areas of productions were estimated for the biodiesel manufacture of the 10.000t a year capacity. Calculating all the cost elements for the manufacturing plant of 10.000t capacity, the authors determined the biodiesel price to be 53,21 dinars/kg or 46,99 dinars/l. When the price is increased for the accumulation value of 10% as common in our practice, the obtained biodiesel price of 51,69 dinars/l is still lower than market price of D2 fuel. Therefore, the authors ascertained that the biodiesel produced from rapeseed was competitive with the standard diesel fuel but also they indicated the issues related to exploitation characteristics as well as the development of distribution network.

Lately, biodiesel production from castor oil has been researched since the castor yield is 80-100% higher than other oilseeds (sunflower, soya, rapeseed) whereas the obtained product is of a high quality and wide range of usage (Samardžija et.al. 2008; Premović, 2011).

Results of numerous economic analyses indicate the important role of the state in price forming of biodiesel. Introducing of suitable incentives, reduction or tax exemption in biodiesel production, providing guarantees for loans, can help to achieve competitive price of biodiesel on the fuel market and thus to enable great use of this energy source (Jovanović et.al. 2005; Furman et.al. 2006).

In order to reduce the cast of biodiesel production process, it is necessary to achieve optimal technological solution and to permanently improve it. Results of numerous researches indicate that one option of reducing the biodiesel production costs is to apply a continuous process of transesterification which also reduces the period of time of esterification process and increases production capacity. Production of high quality glycerol is another way to reduce the costs of biodiesel production. In case of inability of raw glycerol market valorisation, it is justified to use glycerol as the fuel for thermal energy. Maximal efficiency in biodiesel production entails valorisation of all the obtained by-products such as the pressed cake obtained in the vegetable oil production having multiple usage especially in food industry or potassium sulphate obtained in transesterification of fats (Furman et.al. 2005, 2006).

Biodiesel can be produced from waste fats from slaughter industry or rendering processing. Used edible oil as a raw material for biodiesel production is another way to

considerably reduce the cost of biodiesel production (Predojević and Bošković, 2009; Premović, T., 2011).

Compared to raw vegetable oils, price of used – waste vegetable oils is lower for about 50-60% depending on their source and availability (Rice, 2000; Predojević and Bošković, 2009). Using waste oils to produce biodiesel contributes not only to reduction of biodiesel production price but represents a contribution to environment protection for solving the problem of such waste disposal. Generally, these oils end up in sewage causing the problem of waste water treatment (additional investment in energy consumption) or they integrate into the food chain in the production of animal food becoming a potential cause for health problems in people (Predojević and Bošković, 2009; Premović, T., 2011).

Certain countries in Europe researched the possibility of waste oil collecting and the results of researches conducted in seven countries indicated that it was possible to collect 0.7 - 1 Mt of waste oils (Gonzalez-Gomez et al., 2002). However, the amount of waste oils is limited, production capacities are low and additional processing of raw material is recommended due to poor characteristics compared to the fresh unused edible vegetable oils having a greater content of free fatty acids, water, hydro peroxide, carbonyl and polymeric components of great molecule mass (Predojević and Bošković, 2009). Considering that quality of raw material influences on characteristics of produced biodiesel, raw material is limited in content with free fatty acids to maximal 0.5% mas. and with water to maximal 0.3% mas. Namely, present fatty acids and water participate in secondary reactions of saponification, hydrolysis and neutralization forming soaps and gels (emulsions) which hardens separation and purification of fatty acid methyl ester mixture and reduces the yield of the product (Hana and Ma, 1999; Predojević and Bošković, 2009). Purification process of obtained methyl esters after removing the excess methanol and glycerol is very important and mostly is conducted using water, citric and sulphuric acid or silica gel but also in different ways (e.g. using warm distilled water, 5% phosphorus acid, etc.) (Šiler-Marinković and Tomašević, 1998; Predojević and Bošković, 2009).

Possibilities of biodiesel production in Serbia

Serbia disposes of significant land potential for oilseed production used in biodiesel processing. Considering oil content in seeds and its price, rapeseed is the most optimal raw material for biodiesel in our region. Domestic oil refineries also have a potential to play the key role in providing future drives for biodiesel production (Kiš et.al. 2007).

However, biodiesel obtained from fats is more expensive than diesel obtained from oil. In order to be an adequate and sustainable substitute for fossil fuels, biodiesel must be economically competitive to fossil fuel and therefore, it is necessary to determine and analyse all the factors which could improve production and use of biodiesel through reduction of biodiesel price (Ma and Hanna, 1999; Jovanović and Zekić, 2007; Premović, T., 2011).

Oilseeds in Serbia are cultivated on about 270.000 ha, with the potential of over 620.000 ha. Depending on the cropping plan with a potential seedbed of 350.000 ha, it is possible to provide raw material for production of 212.800 to 250.600 t biodiesel.

Sunflower, soya and rapeseed are considered as most important oilseeds in our region. In Serbia they are cultivated on 8.85% of arable land, or on 30.26% of total area with industrial plants in the country (Kiš et.al. 2007). Analysis of regional characteristics of most important oilseed production indicates the possibility to spread these crops,

especially in Central Serbia, where they engage only 1% of arable land. Therefore, further enlarging of oilseed area could and should be directed to the region of Central Serbia, primarily of rapeseed, considering that this oilseed has no demands in quality land and grows on altitude up to 700m, but also of soya in river valleys.

Serbia, thus, disposes of considerable land potential for oilseed production used in biodiesel processing. Additionally, domestic oil refineries also have a potential to play the key role in providing future drives for biodiesel production. Namely, it is known that processing capacities of refineries are considerably over-dimensioned for about 190.000 t, compared to domestic needs in raw oil. Existent market, economic and political circumstances determine whether the excess in production-processing of oilseeds is to be refined (edible oil), exported or processed into biodiesel (Kiš et.al. 2007).

Also, there is a possibility for existent refineries to spread their drives for biodiesel production. Thus, for example, if a drive of 10.000t capacity were built, the amount of total investments would be 4.450.705 euros. Investment in transesterification equipment is estimated as the greatest issue in the investment structure ranging from 3.200.000 to 5.100.000 euros and depending on the production capacity (Kiš et.al. 2007).

Conclusions

Advantages of biodiesel production and use in comparison to oil and other energy sources, from the aspect of sustainable agriculture function, are numerous and could be observed in different ways. According to the results of various researches and our personal research and analyses, we emphasize following advantages of biodiesel use from ecological, economic, energy and technical aspect:

1. ecological aspect: use of biodiesel globally influences on reduction of greenhouse gas, particle and aromatics emission: CO, CO₂, SO₂, soot, benzene, toluene. It is nontoxic and biodegradable – fossil diesel is dissolved only 50% during first 21 days after spilling whereas biodiesel is dissolved 98% without consequences for the same time. B100 reduces the risk of cancer for 94% and B20 for 27%.
2. economic aspect: on macroeconomic level, development of biodiesel production would be caused by the influence on the following indicators:
 - ✓ employment,
 - ✓ increase of industrial production,
 - ✓ additional overflow of funds into agriculture,
 - ✓ contribution to economic growth of rural areas,
 - ✓ reduction of macroeconomic factors dependence on external factors,
 - ✓ industrial development would be enabled through progressive attitude of the state in terms of:
 - subsidies policy,
 - tax policy,
 - long-term strategy on energy resource management.
3. technical aspect: biodiesel of EN 14214 quality represents fuel of high quality for diesel engines. Biodiesel specifications are similar to the regular diesel whereas the improvement is in the oxygen content in biodiesel providing a better combustion process and leading to reduction of exhaust gases emission and improvement of engine

lubricity resulting in better efficiency and durability. These factors partly compensate the effect of low energy content, better preservation and handling.

4. energy aspect can be sublimed into two facts: biodiesel is a renewable energy source and its use reduces the need for fossil diesel which preserves stocks and reduces the risk of supply (Košut et.al. 2008).

Therefore, considering European policy related to biofuels, especially biodiesel (energy, agriculture, research projects, etc.), critical factors of successful implementation can be summed in the following way (Košut et.al. 2008):

- price of agricultural products which are not used in food industry and forming of new markets,
- availability of arable land for the production of industrial plantations of oil crops,
- tax benefits enabling for biodiesel to be competitive to fossil diesel,
- long-term distinct participation of the state through establishing legislative framework and referential goals to introduce biofuels,
- promotional pilot projects implemented on local, regional and national level.

According to the previous knowledge, it is concluded that technological development provided biodiesel production from all fats of vegetable and animal origin and thus started the race to conquer raw material base. Oilseed plants represent the greatest potential in biodiesel production. In order to obtain larger quantities of raw material, optimal production conditions are imposed on potential producers. Upcoming new generation of technology for biodiesel production will be much profitable and productive with widening of potential raw material types. Substitution of mineral fuels with biodiesel is expected to exceed 20% in several countries. At the same time, biodiesel economy must be directed towards sustainable development whereas people's appetite for energy must not further uncontrolledly increase for biodiesel and biofuels in general will supplement instead of replacing oil fuels. Expanding the share of biodiesel in total consumption of fuel for transport and agriculture could cause basic changes in the global history of energy (Premović, T., Milićević, Z. 2015).

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**THE IMPACT OF THE INCOME FROM THE PLASTIC TUNNEL
PRODUCTION OF TOMATOES, PEPPERS AND CUCUMBERS OVER
THE FAMILY BUDGET OF THE SELECTED AGRICULTURAL
HOUSEHOLDS**

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Abstract

Considering the general principle for the area under selected crops to be higher than 0.02 ha, 25 family agricultural households from the Pcinja district are included in the survey. In that context, the survey captures 8 agricultural households from the municipality of Vranje, 5 from the municipality of Bujanovac, 4 from the municipality of Presevo, 3 from Vladicin Han, 2 agricultural households from the municipalities of Trgoviste and Surdulica in particular, as well as 1 from the municipality of Bosilegrad. The total income generated from non-agricultural activities (salary, pension, social aid, inflow from relatives abroad, etc.), as well as the agricultural income are determined. The agricultural income is consisted of two parts, i.e. the value of the agricultural production determined by farm-gate prices for the period 2008-2010 on one side, and the value of the direct support to the farm production (subsidies), on the other side. The results reveal that the total income is between 7,687.92 EUR for the T.H. agricultural household in the municipality of Bujanovac to 32,240.19 EUR for the S.D. agricultural household from the same municipality. The average income is 16,706.9 EUR, whereas the agricultural income participates with 86.2%. Out of the total agricultural income, the income generated from crop production has a share of 86.8%. The share of the income by selected crops in an average is determined as: 18.83% for the tomato production, 11.83% for pepper production and 17.56% for production of cucumbers. The plastic tunnel production generates income in an average of 45.21% from the total family budget of the analyzed agricultural households.

Key words: family agricultural households, income, plastic tunnel, tomatoes, peppers, cucumbers.

Introduction

In conditions with heterogeneous assortment of cultures, where the production structure includes number of crops and different types of livestock, the decision of which crop or livestock type will dominate the total engaged area is of great importance. The significance

of this decision is even larger if the crop production includes intensive crops such as vegetable crops and especially crops cultivated in greenhouses. It is a known fact that this kind of cultivation on a relatively small areas provide relatively high production yield, which is valued differently on the market. The difference in the valuation provokes different effects on the amount of income for the households. In general, the revenues depend on the type of the crop type, i.e. the product. The revenues from the same product dependent also on the market conditions, the time of their delivery (when they are marketed), and to a large extent depend on their quality. Namely, there is a difference when the market has more supply than demanded, in which conditions the product is sold at a lower price than when the demand is greater than the supply. Correspondingly, the products are sold at a relatively higher price when the product in the winter and spring months, opposite of the products' prices in the summer and autumn months. Moreover, the product produced by the principles of organic food production should also reach higher price over the products produced on the conventional manner. Bulatović (1997) performed research of the economic and production factors in the production of pepper on a private property in the Skadar basin region in Montenegro. The research confirmed that the consumption of human labor is 1832 h/ha on an average area of 0.86 ha, and the average yield is 26.10 t/ha. Labor productivity, expressed through the value of production amounted to 89.00RSD/h. The amount of human labor in the pepper production, for an average yield of 15,100 kg/ha, is 1798 h/ha on average (Bošnjak Danica, Jovanović M., 1997). In these conditions, as well as use of 57 tractor hours, the labor productivity amounts 8.4 kg/h or 7.0 min/kg on average. Живковић D. (2007) and associates performed analysis of the organizational-economic parameters in the production of pepper in the case of the rural households. Among other things, the authors concluded that pepper is a relatively highly labour intensive crop, with low level of mechanization in the production processes. In fact, they found that 1,116.9 hours of human labour, and the intensity level of 7.69 is spent for one hectare. For production of one tone of peppers 27.9 hours of human labour and 3.63 hours of machines is spent. The yield was 40 t / ha in 2005, and the value of production at an average selling price of 25,00 RSD/ kg amounted one million dinars. According to the authors, the level of efficiency was 3.16 and the coverage of the variable costs 216.4%.

Peševski and Stojkov (2014) performed comparative analyses on the amount of variable costs and gross margin in the production of pepper in greenhouses and outdoors in Strumica, Macedonia. The authors found that the variable cost in the production of pepper in greenhouses amounted 3,073,773.00 MKD/ha and the gross margin amounted 388,150.00 MKD/ha, while the variable costs in the outdoors production of pepper amounted 4,526,260.00 MKD/ha and the gross margin was 348,755.00 MKD/ha. Pavlović N. et al. (2010) found that the total profit from the organic production of tomatoes produced in Vojvodina (Serbia) amounted to 6,210 EUR/ha, with a 41.4% rate of return and 1.71 cost ratio. Whereas for the production of peppers in the same conditions, the profit amounted to 6,110 EUR/ha, 43.33% rate of return and cost ratio of 1.76. According to Tsoho and Salau (2012), the total cost of tomato production consist of two components: fixed and variable. Variable costs dominate the total cost of production. In circumstances where the household rents land, these costs participate with 92.19% share on average, while when using its own land - 87.89% of the total costs. For Morteza, Ajabshirchi, Mobtaker and Abdi (2012) the focus of the research was on the energy consumption, and

cost-benefit analysis of production. They found that the total cost of production of tomatoes, with an average yield of 135,000 kg, amounted to 34,939 \$/ha, and the rate of gross margin 75.9%. The total cost in the cucumber production, with an average yield of 88,123 kg, amounted to 31,956 \$/ha, and 65.1% rate of gross margin. The art of the household members is to choose from a number of vegetables that will provide the highest income and participate with the highest share in the family budget. Therefore, the purpose of this paper is to examine the impact of the revenues from the three different kinds of vegetables grown in greenhouses, on the family budget of selected farms in the Pčinja district in R. Serbia.

Materials and methods

In order to realize the goal of this research, 25 households were surveyed. The selected households were of mixed type, with vegetable and livestock production producing among other things, tomatoes, peppers and cucumbers in greenhouses. The questionnaire was constructed in a way that can easily identify any family income and variable production costs. The data was then processed using mathematical and statistical methods common for such research. The results are presented in tabular and graphical manner.

Results and discussion

The total revenue in the selected group of family farms range between 7,687.92 EUR, in the T.N household from the Bujanovac municipality, and 32,240.19 EUR in the S.D household from the same municipality, and the income with a median value was 16,706.9 EUR. Here, agriculture accounts for 86.2%, with 86.8% of the production devoted to crop production. The family farm in the municipality of Bosilegrad (TN) expressed the lowest income of the agricultural sector (9,934.8 EUR), although with relatively large amount of arable land (3.56 ha). Besides the land cultivation, the household also owns animals (2.67 pigs cows and 2). Accordingly, parallel to the intensive plastic tunnel production the production structure includes alfalfa and potato. In regards to the previously set objectives in the survey, it is apparent that the earnings from the selected crops are important. The revenue share of the tomato production, account for 15.83% of the total income on average in the investigated group of farms. The lowest share of these revenues (6.82%) is observed in the N.B household from the Bosilegrad municipality, and the A.M household from the Bujanovac municipality recorded the highest share (34.28%) (Figure 1).

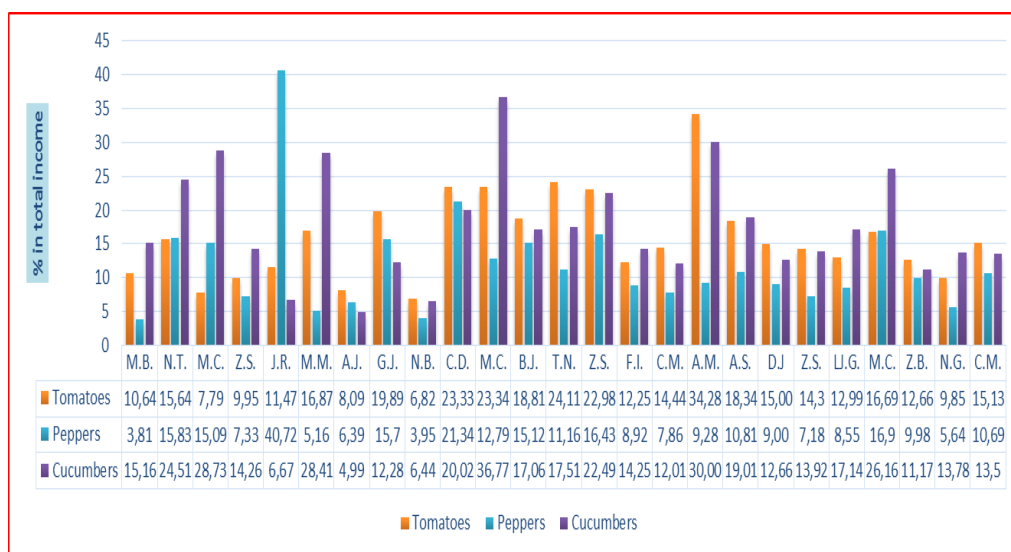


Figure 1 – Share of the income from the selected crops in the total income of the households

The lowest share of income from the production of peppers (3.81%) was registered in the M.V household in the Vranje municipality, and the highest (40.72%) share was seen in the case of the J.R household, again in the municipality of Vranje.

The generated revenues from the production of cucumbers, participate with 17.56% of the total household income on average. The minimum value (4.99%) in the relative share of revenue from cucumbers is seen in the family farm A.J in the municipality of Vranje, and the maximum value (36.77%) in family farm M.C. in the municipality of Bujanovac.

The profitability of the production, as the ratio between the output and the variable costs, shows the generated production for a dinar of variable costs spent in the process of production, expressed in monetary units. Profitability, or the profitability indicator determine the relative share of income in the value of production. Based on the concept of gross margin we calculate the degree (ratio) of variable costs coverage with the amount of gross margin in the case of selected crops.

The highest degree of rational labor and resource use is seen in the production of tomatoes in the S.D household, in the Bujanovac municipality; where one dinar of variable costs generates a value of 4.3 RSD (Table 1). Contrary to this situation, two farms (M.S and Z.S) articulate minimal profitability and investment of human labor. Overall, in the case of 11 households from the total of 25 households, the profitability is higher than the average.

Table 1. Comparative outlook of the qualitative economic indicators in the production of tomatos, peppers and cucumbers

Household	Tomatoes			Peppers			Cucumbers		
	Economy	Profitability	Coverage	Economy	Profitability	Coverage	Economy	Profitability	Coverage
M.B.	2,6	61,9	162,2	2,5	59,6	147,4	3,0	108,84	327,99
N.T.	4,2	76,1	318,9	2,2	54,0	117,6	2,4	35,35	84,66
M.S.	1,6	35,9	56,0	2,1	65,3	139,8	2,8	36,61	102,25
Z.S.	1,6	35,7	55,5	1,5	34,8	53,4	2,8	33,07	91,15
J.P.	3,0	66,8	201,0	4,4	77,4	342,8	3,1	40,37	126,62
M.M.	2,8	64,3	180,3	2,4	58,0	138,0	2,6	125,73	324,50
A.J.	3,1	68,2	214,2	2,2	54,1	117,7	5,0	36,75	185,08
G.J.	2,0	49,5	98,0	2,3	55,9	126,6	1,7	53,12	88,53
N.B.	2,3	56,0	127,4	1,6	39,3	64,8	2,2	60,77	133,95
S.D.	4,3	77,0	333,9	4,6	78,3	361,8	2,7	47,76	128,99
M.C.	2,4	58,7	142,3	2,9	65,4	189,1	1,8	32,16	58,20
B.J.	2,0	50,9	103,7	2,9	65,7	191,1	1,4	21,10	30,39
T.N.	2,1	52,0	108,2	1,5	31,5	46,0	2,7	34,96	95,76
Z.S.	2,1	51,8	107,4	2,8	64,1	178,6	2,0	47,89	96,55
F.I.	2,8	64,0	177,7	2,3	57,1	133,1	3,0	82,23	246,60
S.M.	2,2	50,9	109,6	1,6	35,5	55,0	2,3	40,06	93,64
A.M.	2,4	58,7	142,3	2,9	65,4	189,1	1,8	32,16	58,20
A.S.	2,9	65,4	188,7	2,2	55,5	124,8	2,5	62,31	157,22
D.J.	2,9	65,1	186,3	2,3	56,7	130,7	2,6	47,46	125,72
Z.S.	2,8	64,2	179,0	2,0	50,6	102,3	2,3	55,20	127,74
L.J.G.	2,6	61,8	161,9	2,1	52,3	109,8	2,7	94,26	252,41
M.S.	2,3	56,3	128,8	2,5	60,2	151,4	1,7	45,79	77,28
Z.B.	3,5	71,2	247,0	3,5	71,7	253,5	2,6	47,30	121,64
N.G.	2,9	65,4	188,8	2,0	50,1	100,4	2,8	104,06	295,62
S.M.	2,4	57,6	136,1	3,0	66,5	198,1	2,2	43,33	96,35
Legend		Minimum			Maximum				

The highest degree of rationality of the investments of labor and resources in the production of pepper is same as in the case of the tomato production (S.D household), and the lowest level is seen at Z.Shousehold from Vranje municipality of. In general, around 32% of the total agricultural households in this research, have a higher cost than average. In the process of cucumber production, the most rational input spending's was seen in the B.J household from the Vranje municipality, and the lowest level of rational use of inputs were in the Bujanovac municipality in the B.J household. Here, only 7 households have been exercising better results than average productivity (2,4 RSD). Out of the total number of households in the sample, in the case of all of the selected crops the profitability was higher than the average value in 13 households. The best results in the case of the tomato and pepper production was realized by the S.D household from the Bujanovac municipality

and the worst in the tomato production Z.S household (Municipality of Vranje), while the same was the case in the T.N household (Bujanovac municipality) when pepper production was concerned. Concerning the profitability of the cucumber production, the best results are seen in the M.V household from the Vranje municipality, and the worst results were perceived in the B.J household from the Bujanovac municipality. The coverage of the variable costs with the gross margin in the tomato production was averaging 162.2% of the pepper production and 141.1% of the cucumber production. A comparison of the coverage rate in the investigated sample (25 households) shows that the production of tomatoes, the lowest level of coverage (55.5%) was registered in the Z.S household (Vranje municipality) and the highest (333.9%) in the S.D household (Bujanovac municipality). In the case of pepper production, the highest level of coverage (361.8%) was registered in the S.D household from the Bujanovac municipality, and the opposite was seen in the T.N household (Bujanovac municipality) with a minimum amount of coverage of 46%. The coverage of variable costs in the production of cucumbers is the highest in the M.V household from the Vranje municipality, and the lowest with 30.39% in the case of B.J household (Bujanovac municipality).

Conclusions

Based on the comparison of the qualitative indicators we can recommend that the tomato and pepper production should follow the example of S.D. households in the Bujanovac municipality, since this is a household which generated the best economic results. While in the cucumber production the M.V in the municipality of Vranje should be a representative example household, because it achieves the best economic results. In the case of the S.D household, based on percentage shares of revenues from these crops in the total household income, we recommend reducing the area under cucumbers in order to reduce their share from 20.02% in favor of the tomato and pepper production. Recommendation for the M.V household from the Vranje municipality is that in the future it should adapt the structure of sowing on their land property of 2,6 ha, in favor of the plastic tunnel production, especially in the production of peppers, and in order to increase the share of these revenues by 3.81%.

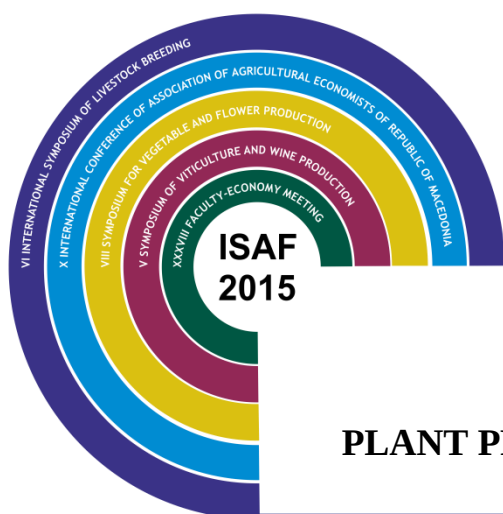
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SECTION 3. PLANT PROTECTION - PHYTOMEDICINE

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INFLUENCE OF SOME HERBICIDES ON BARLEY 1000 KERNEL WEIGHT DEPEND ON GROWTH STAGES

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Abstract

During 2006, 2007 and 2008 an experiment was carried out to investigate influence of six herbicides (2,4-D, MCPP+dicamba, triasulfuron+dicamba, 2,4-D+florasulam, amidosulfuron+jodosulfuron and florasulam+flumetsulam) on 1000 kernel weight of three barley varieties (*reks*, *NS 293* and *egej*), treated in the three different growth stages (tillering, first node visible and second node visible). In 2006 triasulfuron+dicamba applied in tillering growth stage significantly reduce barley 1000 kernel weight at *reks* variety compared with weed free control. MCPP+dicamba in tillering growth stage significantly increase barley 1000 kernel weight at *reks* variety compared with weed free control. Similar, MCPP+dicamba applied in second node stage significantly increase barley 1000 kernel weight at *NS 293* variety compared with weed free control. At *egej* variety florasulam+flumetsulam, 2,4 D+florasulam and triasulfuron+dicamba applied in tillering stage significantly increase barley 1000 kernel weight compared with weed free control. Florasulam+flumetsulam and amidosulfuron+jodosulfuron applied in second node stage significantly increase barley 1000 kernel weight compared with weed free control. In 2007, 2,4 D applied at tillering stage significantly reduce barley 1000 kernel weight of *NS 293* variety compared with weed free control. In 2008, 2,4 D+florasulam applied at first node visible stage significantly increase barley 1000 kernel weight at *reks* variety compared with weed free control. Similar, 2,4-D and 2,4 D+florasulam, as well as 2,4-D+florasulam, amidosulfuron+jodosulfuron and florasulam+flumetsulam applied at tiling stage and the first node visible stage, respectively significantly increase barley 1000 kernel weight at *NS 293* variety compared with weed free control.

Key words: barley varieties, herbicides, growth stages, barley 1000 kernel weight.

Introduction

Barley (*Hordeum sativum* Jessen) in the Republic of Macedonia is grown on about 47 500 ha with average yield of 3 440 kg/ha (Anonymous 2009). It is the second cultivated crop right after the wheat. Very often barley production is disturbed by weed infestation. In barley losses due to competitive effects of weeds estimated at 15-25% of potential production. Contemporary chemical weed control in barley and wheat begins after Second World War. Since then, over 50 a. i. are synthesized for selective weed control in barley and wheat. The most of the herbicides which are used in barley is foliar applied and tillering stage is the optimum growth stage for their application. By using the herbicides in

advance growth stages of barley, sensitivity can be increased and barley yield elements can be reduced (Allien 1966, Markovic 1978, Rinella et al., 2001). Application of herbicides during the different growing stages can have various influences on 1000 kernel weight. Positive influence of some herbicides on 1000 kernel weight was found by Ognanovic et al., (1988). Spasik (1972) emphasized that tested herbicides have negative impact in first and positive in second year of examination. According to this, the aim of the experiment was to evaluate the influence of different herbicides applied in three growth stages on 1000 kernel weight at some barley varieties.

Materials and methods

Field trials were conducted during 2006, 2007 and 2008 at the Agriculture institute in Skopje. The experimental design was randomized complete block system with four replicates, and harvest plot size of 16 m². The studies were carried out with three barley varieties: *reks*, *NS 293* and *egej* which were seeded with seedling rate of 200 kg/ha.

Table 1. Variants of the trial

Variants- active ingredient (a.i.)	Trade names	Rate	Time of application
Weed free control	/	/	/
2,4-D	DMA-6	1 L/ha	I, II, III
MCPP+dicamba	Banvel-P	4 L/ha	I, II, III
Triasulfurone+dicamba	Lintur	150 g/ha	I, II, III
2,4-D+florasulam	Mustang	0,5 L/ha	I, II, III
Amidosulfurone +jodosulfuron	Sekator	0,25 L/ha	I, II, III
Florasulam+flumetsulam	Derby	60 ml/ha	I, II, III

I-tillering stage

II- the first node stage

III- the second node stage

All herbicides were applied with CO₂-pressurized backpack sprayer with 300L/ha water. After harvesting, barley 1000 kernel weight was measured in seed testing laboratory of Agricultural institute in Skopje. The examinations were determined based on 100 randomly selected spikes of each repetition i.e. 400 spikes of each variant. Barley 1000 kernel weight was measured according to ISTA methods. The data were subjected to statistical analysis applying LSD-test

Results and discussion

In 2006 (Table 2) barley 1000 kernel weight was ranged from 42,3 g at *reks* variety treated with triasulfurone+dicamba at tillering stage to 51,8 g at *NS 293* variety treated with MCPP+dicamba at the second node. Highly significant decrease of barley 1000 kernel weight ($p < 0,01$) compared with weed free control was noted at *reks* variety in tillering growth stage treated with triasulfurone+dicamba. Significant increase of barley 1000 kernel weight ($p < 0,05$) compared with weed free control was found at *reks* variety in tillering stage and at *NS 293* variety in the second node stage treated with MCPP+dicamba and at *egej* variety in tillering stage treated with triasulfurone+dicamba, as well. Highly significant increase of barley 1000 kernel weight ($p < 0,01$) compared with weed free control was

recoded at *egej* variety in the second node stage treated with florasulam+flumetsulam and amidosulfuron+jodosulfuron and in tillering stage treated with florasulam+flumetsulam and 2,4-D+florasulam.

Table 2. Influence of herbicides on barley 1000 kernel weight (2006)

Variants	Varieties								
	Reks			NS 293			Egej		
	Tillering	First node	Second node	Tillering	First node	Second node	tillering	First node	Second node
	g	g	g	g	g	g	g	g	g
Weed free control	46,8	46,8	46,8	48,0	48,0	48,0	45,3	45,3	45,3
2,4-D	47,8	49,5	49,5	49,3	49,5	48,3	46,3	48,8	47,8
MCP+dicamba	51,0*	45,8	45,8	47,8	47,5	51,8*	47,0	46,9	47,9
Triasufurone +dicamba	42,3**	49,3	49,3	50,5	48,0	48,5	48,0*	48,6	47,2
2,4-D+florasulam	49,8	47,8	47,8	49,5	50,8	48,8	49,5**	48,7	48,2
Amidosulfuron+jodosulfuron	44,0	46,0	46,0	50,5	47,8	51,0	47,5	46,6	48,3**
Florasulam+flumetsulam	48,3	47,0	47,0	46,3	51,8	49,3	49,8**	49,0	48,8**
LSD 0.05	3,2	4,4	4,1	4,1	4,1	3,6	2,5	3,3	2,6
LSD 0.01	4,4	6,0	5,5	5,6	5,5	4,9	3,3	4,5	3,5

In 2007 (Table 3) barley 1000 kernel weight was ranged from 46,5 g at *reks* variety treated with 2,4-D in tillering stage and triasufurone+dicamba in tillering and the first node stage to 50,8 g at NS 293 treated with MCP+dicamba in the first and second node stage and 2,4-D in the first node stage, respectively. Significant decrease of barley 1000 kernel weight ($p<0,05$) compared with weed free control was found only at NS 293 variety in tillering growth stage treated with 2,4-D.

Table 3. Influence of herbicides on barley 1000 kernel weight (2007)

Variants	Varieties								
	Reks			NS 293			Egej		
	Tillering	First node	Second node	Tillering	First node	Second node	Tillering	First node	Second node
	g	g	g	g	g	g	g	g	g
Weed free control	47,5	47,5	47,5	50,8	50,8	50,8	49,0	49,0	49,0
2,4-D	46,5	48,3	48,8	48,3*	50,8	49,5	49,5	50,5	50,7
MCP+dicamba	47,8	47,5	47,3	50,5	50,8	50,8	49,3	48,0	48,3
Triasufurone +dicamba	46,5	46,5	47,0	50,0	50,5	50,5	47,8	49,3	48,8
2,4-D+florasulam	47,8	49,3	47,8	50,5	50,3	50,0	50,3	49,5	49,3
Amidosulfuron+jodosulfuron	47,5	47,0	48,8	50,3	49,3	49,5	49,5	50,3	50,0
Florasulam+flumetsulam	47,0	47,5	46,8	48,8	49,3	50,3	49,3	50,7	49,8
LSD 0.05	3,6	2,2	2,2	2,0	2,4	2,6	2,5	2,5	2,4
LSD 0.01	4,9	3,0	3,0	2,7	3,2	3,5	3,4	3,4	3,2

In 2008 (table 4) barley 1000 kernel weight was ranged from 47,8 g at *egej* variety treated with MCPP+dicamba in first node stage to 52,5 g at *reks* variety treated with 2,4-D and MCPP+dicamba in tillering stage and 2,4-D+florasulam treated in first node stage.

Table 4. Influence of herbicides on barley 1000 kernel weight (2008)

Variants	Varieties								
	Reks			Ns 293			Egej		
	Tillering	First node	Second node	Tillering	First node	Second node	Tillering	First node	Second node
	g	g	g	g	g	g	g	g	g
Weed free control	50,3	50,3	50,3	50,3	50,3	50,3	49,3	49,3	49,3
2,4-D	52,5	51,0	51,3	52,3*	51,3	51,5	49,8	48,8	48,5
MCPP+dicamba	52,5	52,0	50,5	50,8	51,5	51,0	49,3	47,8	48,0
Triasufurone +dicamba	51,5	51,5	51,0	51,3	51,3	50,5	49,0	48,3	48,3
2,4-D+florasulam	52,0	52,5*	50,8	52,3*	52,0*	52,0	49,0	50,5	49,3
Amidosulfuron+jodosulfuron	52,3	52,0	51,8	52,0	52,3*	51,0	49,8	48,8	48,8
Florasulam+flumetsulam	51,3	51,0	51,5	51,8	52,0*	51,3	49,5	49,8	48,5
LSD 0.05	2,4	2,0	2,3	1,9	1,6	1,8	1,7	2,4	1,8
LSD 0.01	3,3	2,7	3,1	2,6	2,2	2,4	2,4	3,3	2,5

Significant increase of barley 1000 kernel weight ($p < 0,05$) compared with weed free control was found at *reks* variety in the first node stage treated with 2,4-D+florasulam and at NS 293 in tillering stage treated with 2,4-D and 2,4-D+florasulam, also, in first node stage treated with 2,4-D+florasulam, amidosulfuron+jodosulfuron and florasulam+flumetsulam.

Differences between growth stages during the herbicide applications in all three years of testing (Table 5) were very small, so they have no impact on barley 1000 kernel weight. Similar results were reported by Pacanoski (2003).

Table 5. Influence of growth stages on barley 1000 kernel weight

	Average of all herbicides								
	2006			2007			2008		
	Reks	NS 293	egej	Reks	NS 293	Egej	Reks	NS 293	Egej
	g	g	g	g	g	g	g	g	g
tillering	47,2	49,0	48,0	47,2	49,7	49,3	52,0	51,7	49,4
first node visible	47,5	49,2	47,5	47,7	50,1	49,7	51,7	51,7	49,0
second node visible	46,7	49,6	47,8	47,7	50,1	49,5	51,1	51,2	48,5

According Quinby et al., (1996) dicamba applied in wheat in booting and flowering stage increase and applied in tillering stage decrease 1000 kernel weight. Lalluka (1976)

found that MCPP+dicamba applied in tillering and flag leaf emerge stage only at *Otra* variety decrease 1000 kernel weight.

Conclusion

Based of the obtained results it can be concluded that the influence of herbicides on barley 1000 kernel weight is not dependent on the growth stages. If conditions do not allow herbicides to be used in the optimal period, it can be used no later than second node stage, without having a negative impact on the barley 1000 kernel weight.

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STATE OF THE INTEGRATED PEST CONTROL SYSTEMS IN THE VINEYARDS IN BULGARIA

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Abstract

The application of modern methods and practices in the diagnostics and pest control is not only an objective, but a means for the achievement of sustainable agricultural development. In line with the European legislation and the adopted in Bulgaria Plant Protection Act, the compliance with the general principles of integrated pest management has become mandatory for farmers since 2014. The integrated pest management is related to a more focused implementation of available measures for pest control, reducing the dependency on the use of pesticides, priority application of biological, physical and non-chemical methods, means and plant protection products with a low risk to human health, animals and the environment. Data from studies of bulgarian and foreign scientists concerning the integrated control of diseases, pests and weeds in vineyards, including sanitary selection, the influence of various pests and pesticides on vine, grapes and wine are presented.

Key words: vine, diseases, pests, weeds, integrated control.

Review

Production of high and sustainable yields of agricultural crops is impossible without their protection from diseases, pests and weeds. Therefore various methods and means of prevention and control are applied.

In accordance with Article 14 of Directive 2009/128/EC and Article 55 of EC Regulation 1107/2009 the compliance with the general principles of integrated pest management in crops became mandatory requirement since 2014. The general principles of integrated pest management and the National Action Plan for the sustainable use of pesticides are set forth in the Plant Protection Act, promulgated in SG, issue 61 of 25.07.2014. It is expected the result to be a more targeted use of all available measures for pest control, including pesticides and to contribute to reducing the risks to human health and the environment and the dependency on the pesticides.

It is also expected the impact of that measure to enhance and keep the interest of the farmers in the integrated production methods; to prevent pests to develop resistance to PPPs; to decrease the cost of the farmers for PPPs; to reduce the risks to human health and the environment. The introduction of the mandatory application of the integrated production general principles justifies the targeted research and development efforts related to pest control and management in vineyards. The available gene fund with great genetic diversity is potential for studying the effects of different pests and pesticides on the vine,

grapes and wine in response to the constantly increasing consumers demands for organic foodstuff.

Researches on the issues of vine sanitary selection in Bulgaria began in the late 1960s at the Institute of Viticulture and Enology - Pleven. In the early 1970s the first methodology and scheme for vine individual clonal selection for obtaining healthy planting material were worked out and published (Ivanov et al., 1972; Ivanov et al., 1972a), that on the basis of ongoing studies were periodically updated (Abrasheva et al., 1993; Abrasheva et al., 2004). In the 1980s, the first mother plantations for super-elite (basic) and elite (certified) vine propagation material were established by the methods of clonal and sanitary selection. In 2000, the renovation of the mother plantation at the Institute was initiated as in parallel with the investigations on diseases carried by the planting material researches were aimed at studying the European legislation and sanitary selection schemes of the leading viticultural countries in Europe. In the EU vine planting material categories are defined by Directive 68/193/EEC, as amended by Directive 2002/11/EC and Directive 2005/43/EC. The category of prebasic material was introduced to the existing categories of basic, certified and standard planting material. The amendments in the regulatory framework of the EU and the country's accession to the European Union led to changing and harmonization of the Bulgarian legislation therefore a national certification system for vine planting material was established. In Bulgaria this is regulated by the Seeds and Planting Material Act (prom. SG. issue 20/04.03.2003) and Ordinance No. 95 of 04.08.2006 on trading of vine planting material.

The diseases in Bulgaria object of obligatory sanitary selection are *Grapevine fanleaf virus* (GFLV), *Arabis mosaic virus* (ArMV); *Grapevine leafroll virus* (GLRaV), complex of rugose wood (GVA and GVB); *Grapevine fleck virus* (GFkV); crown gall *Agrobacterium* spp. and dead-arm *Phomopsis viticola*.

Of the viruses investigated in Bulgaria, GFkV, the causative agent of fleck disease has relatively the highest level of incidence (24.81 %) (Genov et al., 2009). Similar results on GFkV incidence in Bulgaria were obtained by Kamenova *et al.* (2007). The high rate of its distribution is caused by the latent course of the disease in the European and some American grape varieties. Furthermore GFkV was not until recently included in the list of viruses for which laboratory diagnostic methods were applied due to its identification at a later stage and respectively obtaining of antibodies for diagnostics (Boscia et al., 1994). In accordance with Directive 2005/43/EC and Ordinance 95 it is obligatory only the rootstocks to be tested for GFkV, and that is a precondition for its further development with the propagation material. Second in distribution is GLRaV-3 (13.83 %). The most severely infected are Druzhba, Storgozia and Rubin varieties due to the fact that they do not show the symptoms typical for the disease. Smaller share belongs to GLRaV-1 (2.64%) and GFLV (1.45%), while *Arabis mosaic virus* - ArMV) was not found in any of the tested vines. GVA (3.23%) was distributed mainly in mixed infections with GLRaV and GFkV (Genov *et al.*, 2009).

Phytoplasmas are prokaryotic microorganisms, belonging to domain *Bacteria*; phylum *Tenericutes*; class *Mollicutes*; order *Acholeplasmatales*, family *Acholeplasmataceae*; genus: '*Candidatus* Phytoplasma' (IRPCM, 2004). Phytoplasmas are localized in the phloem plant tissue and are carried by insects sucking juice. Vine diseases caused by phytoplasmas are a complex disease generally called Grapevine yellows. They are associated with genetically different phytoplasmas of which the most important are *Flavescence dorée* and *Bois noir* (Casati et al., 2012).

Flavescence dorée (FD) is the most important and devastating phytoplasma vine disease. When the vector is not controlled, the number of infected vines can reach 80-100% for several years (Smith et al., 1997). In France and Corsica large areas have been affected and that process is still ongoing, in spite of the mandatory measures for uprooting the diseased vines and vector control using insecticides (Smith et al., 1997; Steffek et al., 2007). In the area of Aleksandrovac, Serbia more than 800 ha of vineyards were affected and eradicated. Losses on investments reached 3.2 million Euro. The lost profits of the producers from the reduced quantities of wine were much more (Steffek et al., 2007).

Vine varieties play an important role on the rate of the infected cicadas in vineyards and thus have an impact on the severity of the disease. The sensitivity of the varieties to FD varies greatly. Chardonnay, Pinot Noir, Pinot Gris, Cabernet Franc, Cabernet Sauvignon, Berbera, Sangiovese, Soave, Prosecco varieties are sensitive while other varieties such as Merlot, Sauvignon Blanc and Syrah are more resistant (Steffek et al., 2007).

The causative agent of *Bois noir* (BN) belongs to 16SrXII-A subgroup of stolbur (Bertaccini et al., 2014). Phytoplasmas from this subgroup attack a wide range of hosts, such as vegetables, tomatoes, potatoes, carrots; field crops - beet and some weeds. BN is transmitted to grapevine by the planthopper *Hyalesthes obsoletus* Signoret (Homoptera, Cixiidae) (Sforza, et al. 1998). Bindweed (*Convolvulus arvensis* L.) and common nettle (*Urtica dioica* L.) are considered the main reservoir of infection in European vineyards. This insect is polyphagous and prefers grassy weeds more than vine. *H. obsoletus* hibernates in the nymph stage and only perennial plant species can serve as hosts. However, adults can be found on many other plant species. Since the vector feeds incidentally on vine (Sforza, et al. 1998), BN spreads more slowly than FD.

From grapevine yellows in Bulgaria so far only Bois noir (BN) has been found (Avramov et al., 2008; EPPO, 2006; Genov, 2012a; Sakaliev et al., 2007), while the causative agent '*Ca. Phytoplasma solani*' was reported also for blackberry (*Rubus fruticosus*) and maize (*Zea mays* L.) (Bobev et al, 2013; Genov et al., 2014). *H. obsoletus* and *Reptalus* spp. are found in Bulgaria as vectors of BN on vine (Avramov et al., 2011). So far FD has not been established, but the presence of its vector - the cicada *Scaphoideus titanus* Ball was registered (Avramov et al., 2011). Furthermore, FD and its vector are available in close proximity to Bulgaria, in Serbia at least since 2003 (Duduk et al., 2003; Duduk et al., 2004; Magud and Tosevski, 2004), where it caused serious damage to wine producers. There is a real risk of FD incidence in Bulgaria, causing epidemics in vineyards.

Crown gall causes huge damages to viticulture worldwide, as its incidence is the greatest in areas characterized by unfavorable climatic conditions for vine in the winter-spring period. The disease has been reported from Europe, North and South America, the Middle East, Australia, South Africa, Japan and China. In our country, the disease is spread in all viticultural regions (Genov, 2012). Following the wide development of the disease during the period 1966 - 1975 the dying of 4600 ha of fruit-bearing vineyards has been registered (Malenin, 1996). The minimum negative temperatures during the months of February, March and April are of utmost importance for the crown gall development Bulgaria. The amount of rainfall, temperature and relative humidity, have the strongest effect in May and June (Genov, 2007). There are no officially registered chemicals for control as the systemic survival of the agent in the vine conductive system limits their application. The preparations used in the past based on dinitro-orto-crezol (DNOC) are currently prohibited due to their high toxicity, but they have effect only on tumors and do not kill the bacteria in vines.

Measures to control crown gall are especially important in farms for the production of vine propagation material. In this connection, to the methodology for individual clonal selection in vine and the first scheme for obtaining sanitary healthy vine seedlings (Ivanov *et al.*, 1972), including initially only economically important viral diseases at a later stage crown gall and excoriosis were added (Abrasheva *et al.*, 1993; Abrasheva *et al.*, 2004).

The methods and means of diagnostics and identification of the agents, including visual, microbiological, serological and molecular ones as well as the time of their application, have the greatest importance for the efficient control of systematic grape diseases. The control could be conditionally divided into two stages: the first one is from the establishment of the variety (clone) to obtaining and maintaining of prebasic propagation material, which is responsibility of the selection centers or private breeders (Genov *et al.*, 2009) and the second one – official control of phytosanitary services covering re-propagation and marketing of the variety from prebasic to certified material.

Black rot until recently was considered almost unknown vine disease in Bulgaria. It was brought to Europe from North America. In 1885 it was observed in France by Viala and Ravaz, who reported it for the first time in phytopathological literature. This was the most spread and harmful disease in the humid regions of the southern states and along the Gulf of Mexico (Brown, 1956 acc. to Vanev 1963). Later, the disease was found in Italy, Russia, Georgia, Azerbaijan, Ukraine and others.

In countries where black rot is an economically important disease (USA, Canada, etc.) the pathogen is considered to be the fungus *Guignardia bidwellii* (Ellis) Viala & Ravaz with anamorphic *Phyllosticta ampellicida* (Englem.) Van Der Aa = *Phoma uvicola* (Viala et Ravaz). *G. bidwellii* is ascomycetous fungus, forming pseudocetes with cylindrical or club-shaped ascus containing 8 unicellular hyaline (transparent) ascospores (Vanev, 1963; Harizanov *et al.*, 2009).

In Bulgaria the disease is not so widely spread however in certain years of wet weather and very frequent rainfall it may cause significant losses. It attacked most often the vineyards located along the Black Sea coast and those in lowlands, not well aired (Malenin, 2003).

Margaritov (1960) found black rot on vine leaves, shoots and berries in Pomorie region. At the same time the disease was found on ripened berries of Bolgar variety in the region of Pleven. In 1961, limited manifestations of black rot were observed in Pleven, Ruse, Plovdiv and Vidin districts (Vanev, 1963).

Vanev isolated the fungus *Phoma uvicola* (Viala et Ravaz): the isolated sample had black, spherical or slightly flattened pycnidia, 100 to 198 µm in diameter. They had a circular ostiole from which an exudate of picnisporos leaked out. Exposed to air the exudate dried out in the form of yellowish ribbons releasing the picnisporos in water. The size of the spores varied between 5 and 11.5 µm in length and 3.1 to 5 µm in width. There is morphological diversity of spores - with rounded corners and sharp at both ends. In our country the pathogen overwinters in the infested parts as pycnidia. According to Vanev (1963; 1995) the superior form *Guignardia bidwellii* has not been detected in Bulgaria.

In Bulgaria until recently there were no registered licensed plant protection products against black rot. The main practices used were aimed at reducing the size of infection, namely: collection and destroying of all infected parts, spring plowing of attacked vineyards, pruning of infested shoots at rest and their destruction (Kostadinova and Lyubenova, 2011).

In France and Switzerland plant protection products (PPP) with active substances from different groups are applied for the disease control: Strobilurin (trifloxystrobin +

tebuconazole); DMI fungicides (tebuconazole, myclobutanil, flusilazole, penconazole); Dithiocarbamates (metiram, mancozeb) (Kostadinova and Lyubenova, 2011; Malenin 2003).

Wilcox (2003) described the infection of black rot at different temperatures and moisture. The shortest period of spreading the infection was observed at temperatures within the range of 15.5°C to 29°C, for which only 6 to 9 hours were needed and high humidity.

According to Molitor et al., (2012), the incubation period of *G. bidwellii* was strongly influenced by temperature. In this regard there has been developed a pattern according to which, the first symptoms of the disease on the leaves appear after reaching the threshold of 175 cumulative day degrees, calculated on the base of the sum of average daily temperatures between 6 and 24°C from the day after infection. According to the authors full control of the infection development on leaves and clusters may be achieved by the use of strobilurin and pyraclostrobin, even after the expiry of more than 85% of the incubation period.

The early researchers thought that only young above ground parts were susceptible to infection by *G. bidwellii*, i. e., there was age-related resistance, but according to Hoffman et al., (2004), conidia could infect the leaves of all ages and the occurrence of the fruit ontogenetic resistance was not well documented.

According to Hoffman et al., (2002), in the USA the most effective preparations for black rot control are (DMI) fungicides with active substance myclobutanil and tebuconazole (Hoffman and Wilcox, 2002; Hoffman and Wilcox, 2003; Hoffman et al., 2004). Full control of the infection development on leaves and clusters may be achieved by the use of strobilurin and pyraclostrobin, even after the expiry of more than 85% of the incubation period (Molitor et al., 2012).

Vine pests, depending on the degree of infestation, can cause serious damage to the foliage and the grape harvest, which in most cases affect adversely vines resistance to low temperatures and some diseases. The most important economically insects are controlled annually, but together with them there are less familiar pests, that require researches on their phenological development and working out a system for their control (Harizanov et al., 2009).

The problem of vineyard pest control in Bulgaria had been studied by a number of authors Nachev, Kostadinov, Harizanov, Stoeva, Nedyalkov, Harizanova, etc. The biology, ecology, influence of abiotic factors on the multiplication of a large number of pests on vine, their preference to individual varieties had been investigated (Kostadinova et al., 2007). Thresholds of economic injury level (EIL) for the different pests were determined. The integrated control benefits maintaining the biological balance in the vineyards and protecting the vintage and environment from pollution had been established. A large number of chemicals from different groups were registered for control of the harmful insects on the vine. Some of them were toxic to beneficial insects and mites, while others showed insufficient efficacy over the years (Todorova et al., 2000).

The research work on the problems of integrated pest control in Bulgaria began in the 1950-ies at the Institute of Plant Protection, Kostinbrod and the Forest Institute - BAS, Sofia. In viticulture, most of the studies in this area belonged to Prof. Harizanov focused on grape moths. He found more than 50 species of parasitic insects and over 60 predatory insects and acars and demonstrated the positive effect of bacterial products and the parasites of the genus *Trichogramma* for management of these pests (Harizanov, 1986). A number of studies were carried out on the application of pheromone traps in integrated

grapevine moths control by Harizanov (1978), Parvanov and Kostadinov (1979), Kostadinov (1980), Stoeva (1985).

In the studies of Kostadinov (unpublished data) on beneficial insects in grapevine biocenoses in plantations around Pleven it was found 4 species of green lacewings and 4 species of ladybirds as the highest density had the common green lacewing (*Chrysoperla carnea*) and seven-spot ladybird (*Coccinella septempunctata*).

In 2008, pursuant to article 6 para. 2 and para. 3 of the Ordinance No. 15 on the terms and conditions for integrated production of plants and plant products and their marking, and in conjunction with article 8a para. 2 of the Plant Protection Act, the Ministry of Agriculture and Food and the National Plant Protection Service published a Manual on Integrated Pest Management in Vine and Berry Crops and a list of key bio-agents (beneficial insects and acars) in vine was prepared (Harizanov et al., 2008).

A classification of plant protection products was made according to the possibility for their application in the integrated plant protection into three groups:

1. Permitted for use in the integrated production (Green List): non-toxic - causing less than 25% mortality of beneficial species.
2. With limited use in the integrated production (Yellow List): slightly toxic - causing 26% to 50% mortality of beneficial species, moderately toxic - causing 51% to 75% mortality of beneficial species.
3. Prohibited for use in the integrated production (Red List): highly toxic - causing more than 75% mortality of beneficial species.

A research team from the Institute of Viticulture and Enology - Pleven (unpublished data) found that the active substances simoksanil, mancozeb, copper oxychloride, difenoconazole, myclobutanil, triadimenol, penconazole, sulfur and tebuconazole were suitable for integrated disease control in vineyards and imidacloprid and thiamethoxam were toxic to bio-agents and are not suitable for integrated control of pests in vineyards.

An essential condition for carrying out integrated disease and pest management in vine is to achieve control over harmful vegetation in the vineyards. Young vines show the highest susceptibility therefore the efficient weed control is a necessary condition for the establishment of a new vineyard. The first 6-8 weeks of vegetation are particularly important. The competition for uptaking the major biotic factors greatly hinders the normal growth of vines. It was found that the species bindweed (*Convolvulus arvensis* L.), field mustard (*Sinapis arvensis* L.), amaranth (*Amaranthus retroflexus* L.) and twitch grass (*Cynodon dactylon* (L.) Pers.) have much more intensive transpiration than vine. Weeds consume not only more water but also more nutrients from the soil (Boychev, 1980). The high rate of weeds usually results in less developed shoots, smaller clusters mass and a lower yield per hectare (Moulis, 1992). The wide incidence of different annual and especially perennial species can prevent mechanized tillage; can serve as a media for development of the agents of some economically important diseases and pests; can increase the risk of frost and vines destroying by frost (Fetvadhiev et al., 1981; Cousens and Croft, 2000).

Associations of earthed-up type are formed in young vineyards as representatives of the late spring weeds prevail – Common barnyardgrass (*Echinochloa crus-gali* (L.) Beauv.), hairy crabgrass, (*Digitaria sanguinalis* (L.) Scop.), setaria (*Setaria* sp.), amaranth, white amaranth (*Amaranthus blitoides* L.), pigweed (*Portulaca oleracea* L.), Canadian horseweed (*Coniza canadensis* L.), etc. The species of other biological groups are comparatively less spread – wild oat (*Avena fatua* L.), shepherd's purse (*Capsella bursa-*

pastoris (L.) Medic.), chickweed (*Stellaria media* L.), groundsel (*Senecio vulgaris* L.). In some cases there could be perennial rooted and root-sprouting weeds - twitch grass, Johnson grass (*Sorghum halepense* (L.) Pers.), couch grass (*Agropyron repens* (L.), bindweed, thistle (*Sonchus arvensis* L.), etc. (Kolev, 1963; Beuret et Neury, 1987; Moreira, 1994; Encheva, 2003; Tonev et al., 2007).

Herbicides based on simazine have been recommended for their control in the course of decades (Chelebiev, 1980; Portnoy and Vovk, 1991; Chelebiev and Encheva, 2002; Karanikolov and Draganov, 2003). Later, triazine herbicides were gradually replaced by other active substances - trifluralin, oryzalin, oxyfluorfen etc., which even having good performance were not yet sufficiently used in young vineyards (Lange et al., 1970; Tonev, 2000). Pendimethalin, napropamide, s-metolachlor exhibit high herbicidal impact (without harmful effects on the vines) applied immediately after planting (Bordelon, 2011).

At present in Bulgaria the main way for keeping the soil surface clean of weeds in young vineyards (up to 3 years) is a system of treatments - deep autumn plowing and several shallow plowings during the vegetation period. It is a common practice the area inside the rows to be earthed-up manually (Tonev et al., 2007). Chemical weed control is performed only as a preventive measure (by repeatedly applying of glyphosate in the previous year). There are no registered herbicides for controlling the harmful plants during the vegetation season.

Conclusions

In conclusion, the ever-increasing concern for environmental protection and reducing the use of pesticides put forward the implementation of integrated systems for pest control in vineyards, which achieve the production of healthy and quality produce with the lowest possible risk to human health and agro-ecosystems.

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UDC: 635.649-24:632.937.1]:631.544

632.937.1:635.649-24]:631.544

Original scientific paper

IMPACT OF *TRICHODERMA ATROVIRIDE* AND EFFECTIVE MICROORGANISMS THE YIELD OF PEPPER (*CAPSICUM ANNUUM* L.) IN THE GREENHOUSE

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Abstract

The experimental trial with peppers was performed in a protected area - high greenhouse in 2014 on a calcareous chernozem. Early hybrid F1 Blondi was used as material. The aim of the study was to determine the influence of *Trichoderma atroviride* and microbiological fertilizer (effective microorganisms) in fruit number and yield of peppers per plant, as well as the correlation of yield and number of fruits peppers. The average number of fruits per plant was 6.82, with the variant bio fertilizer and *Trichoderma atroviride* was higher (7.18 and the 6.84). The yield per plant was higher in plants treated with *Trichoderma* and microbiological fertilizer to 14:24%, or 5.92%, while the control treatment was measured yield of 664.56 g. However, the variance blocks and the variance blocks treatments were not significant for the number of fruits per plant and yield. The correlation coefficient between the number of fruits and yield per plant was highly positive (0.73*).

Key words: microbiological fertilizer, pepper, greenhouse, yield, *Trichoderma atroviride*.

Introduction

Pepper (*Capsicum annuum* L.) is included in the group of the most significant vegetable cultures, it has a great economic importance and high nutritive and biological value (Gvozdenovic et al., 2007; Cvijanovic et al., 2013).

The plant yield depends on the development of every plant's pheno-phase. The organic fertiliser application and effective microorganisms (EM), results with an increasing growth, a greater luxuriance and general condition of plants. The effective microorganisms application affects the growth of healthy and vital plants, even in the agriecological adverse conditions. By this, organic food is produced (Cvijanovic et al., 2013). According Cvijanovic et al. (2014), by foliar application influences the increase in the number of groups of beneficial microorganisms. During treatment with EMs, the active inoculum inhibira the development of harmful (phytopathogenic) microorganisms and thus provide protection plant during the growing season, or in control of the growth of pathogenic microorganisms, which is exempt application of pesticides. The combination of organic fertilizers and EMs increases the number of them and biochemical activity of the soil, which increases the level of organic matter in the soil, the greater availability of basic nutrients for the plants, the good relationship C:N in the soil, which is the essential

parameters for the higher productivity of land (Misustin, 1975, Cvijanovic et al., 2012). It also enhances the transformation of plant nutrients and increases their availability to plants, encouraged more intensive development of plants in the early stages of growth, increasing parametric of yield and high of yield, non-use of mineral fertilizers and chemical pesticides, increasing economic viability of crop (Đukić et al., 2015). *Trichoderma spp.* has a high adaptability to the environment, particularly because of the high reproducibility and aggressiveness against pathogens (Kar et al., 2006; Samuels et al., 2002). This means faster and more efficient operation and long life. The ability to live in the soil *Trichoderma* fungus colonize and occupy the area around the roots of plants and inhabit in the soil pores filled with air. According to Harman et al (1996, 2006) a unique strain of the fungus with high adaptability to different soil conditions of the environment, protect the root system in soils which already contain pathogenic fungi, stimulates plant growth and uniform reproduction, protects the leaves and fruits of plants from attack *Botrytis* and *Phytophthora*, safe product for humans and insects, natural product which is not genetically modified, has a wide range of effects (without resistance), it is easily applied. After applying in the soil, mycelium and spores are activated and begin to grow and colonize a certain extent (Lewis and Lumsden, 2001). The presence of *Trichoderma* eliminates pathogenic fungi different mechanisms of action and as predators of *Trichoderma* direct attacks pathogenic fungi. Products metabolites, toxic substances harmful effect on pathogenic fungi. Harman et al. (2004) *Trichoderma* presents a physical obstacle, because in symbiosis with plant roots do not allow the penetration of pathogenic hyphae in the root of the plant which is a biological control in the production of Harman et al. (2006). The results of previous studies point out that fungus *Trichoderma* has had a significant influence on germination, growth and development and aerial and hypogeal plant parts of pepper, when used as a treatment of soil or seed. The effects are clear. Application of *Trichoderma* has significantly influenced in the promotion of growth and development of pepper, as well as in preventing the development of mycosis (Djokovic et al., 2012; Galonja-Coghill et al., 2013).

The aim of this work was to determine the number of fruits and yield of pepper per plant, depending on the application of microbiological fertiliser (effective microorganisms) and *Trichoderma atroviride*, on the basis of experimental plot in the plastic covered greenhouse. Therefore, the recommendation could be proposed in a wider production for obtaining a larger number of fruits with high and stable yield from healthy plants without the occurrence of pathogens.

Material and methods

As a material used is an early hybrid F1 Blondi. The experiment used 270 plants hybrids BLONDI purchased the seedlings. Hybrid peppers Blondi is of the highest quality. The main feature is that long remains fresh and well tolerated transport. The fruits are thick-walled, compact consistency, the average weight is between 150-220 g. Early hybrid with very strong roots and a powerful herb, unlimited growth and high yield potential. The fruits are fleshy, solid, square-shaped with 3-4 seminal barrier is very attractive. Ripe fruit changes color from svetožute in a yellow high gloss. It is highly resistant to peak fruit rot (spots). In the greenhouses are recommended cultivation of the two branches with a nosedive regular and continuous fertilization throughout the harvesting season.

The experiment was performed in 2014 on a private farm in the municipality of Senta, Gornji Breg and implemented in higher greenhouse. The experimental design was a randomized complete block design with three replications, where they applied the following treatments: microbiological fertilizer (EM active), which contains 80 different types of active effective microorganisms (*lactic acid bacteria*, *photosynthetic bacteria*, *yeasts*, *actinomycetes*, *mushrooms*). The preparation TIFFI, product composed of spores and the mycelium of fungi living, genetically unmodified, which is composed of the fungus *Trichoderma atroviride* and control variant. The used products are applied according to the manufacturer. Preparations were applied by mixing with the soil seven days prior to sowing and foliar application once a week during the growing season. Growing peppers were the two branches with regular pinching off. Before planting was entered into the soil microbial inoculant (effective microorganisms) in an amount of 40 lha⁻¹, an active inoculum was diluted with 200-400 liters of water. The treatments during the growing season: the manufacturer's recommendation is that the first two months: 2-4 lha⁻¹ we dilute with 300 liters of water per treatment. After two months: 4-6 lha⁻¹ dilute with 600 liters of water per treatment. When the plant has six leaves, the treatment of active inoculum performed regularly every week throughout the leaf area of pepper. Also, seven days before transplanting into the soil fed into the TIFI (*Trichoderma atroviride*) with 1.5-2.0 kg ha⁻¹ soil. During the vegetation treated weekly microbiological fertilizer and *Trichoderma atroviride*. Thus dissolved foliar application preparations were applied to each plant (the whole length of the stem, leaf area throughout the peppers).

In the basic treatment of the plowed good burn the manure in an amount of 15 kg.m⁻². Together with manure is plowed 1/3 of the total amount of complex fertilizers NPK 5:12:20. Remaining quantities of nutrients entered the recharge with the addition of water in the root zone system (fertigation). Growing peppers were the two branches with regular nosedive. The experiment was set in three replications. The peppers is made to a permanent place in the protected area in two bands (Dozet, 2015). The distance between the strips was 80 cm and 40 cm between rows. The distance between plants in a row was 30 cm. From each variant is selected by the ten plants which were coated and which were used during the growing period for analysis. For statistical analysis of the results was used to program GenStat 9.1 (Trial Version) and Statistical 12.0. Data were analyzed using analysis of variance, randomized block system.

Results and discussion

Table 1. Analysis of variance is reflected in the number of fruit with microbiological treatments

Sources of variation	Degrees of freedom	Sum of squares	Center of the square	F-ratio	F-pr
Blocks	2	1.2790	0.6395	2.28	
Treatment	2	0.8318	0.4159	1.49	0.329 ^{ns}
Error	4	1.1200	0.2800		
Total	8	1.1200			

^{ns}no significant

The results of variant analysis for the number of fruits per plant depending on the applied treatment are shown in table 1. It can be concluded that the treatment had no statistically significant effect on the examined trait (Tab. 1).

Table 2. Number of fruits per plant

Treatment	Repetition			Sum	Average
	1	2	3		
Control	6.17	6.90	6.23	19.30	6.43
Trichoderma atroviride	7.30	7.53	5.70	20.53	6.84
Microbial fertilizer	7.00	7.40	7.13	21.53	7.18
Sum	20.47	21.83	19.07	61.37	6.82

The average number of fruits, per plant was 6.82, but the largest number was in the treatment with the microbiological fertilizer application, that is, effective microorganisms. The smallest number of fruits was achieved in the control variant (Tab. 2). The analysis of the yield variance per plant shows that the applied treatment did not have a statistically significant effect on the measured property (Tab. 3).

Table 3. Analysis of variance is reflected in the number of fruit with microbiological treatments

Sources of variation	Degrees of freedom	Sum of squares	Center of the square	F-ratio	F-pr
Blocks	2	7267	3634	0.65	
Treatment	2	13580	6790	1.22	0.385 ^{ns}
Error	4	22192	5548		
Total	8	43039			

^{ns}no significant

Table 4. Yield per plant (g)

Treatment	Repetition			Sum	Average
	1	2	3		
Control	610.0	714.3	669.3	1993.7	664.6
Trichoderma atroviride	674.7	811.7	625.3	2111.7	703.9
Microbial fertilizer	816.7	716.7	744.3	2277.7	759.2
Sum	2101.3	2242.7	2039.0	6383.0	709.2

The average yield per plant was 709.2 g (Tab. 4). The smallest fruit weight per plant was recorded in the first control variant repetition (610.0 g), and the largest in the microbiological fertilizer application (816.7). The average of the largest yield per plant was achieved with microbiological fertilizer (effective microorganisms) application (759.2 g). The yield per plant was higher in plants treated with microbiological fertilizer and Trichoderma for 14.24%, and 5.92%, while the yield of 664.6 g was measured on a control variant. However, the block variance and the treatment variance were not significant in the number of fruits per plant and the yield per plant.

The computed correlation coefficient between the fruit number and the yield per plant was high positive (0.73*).

The result analysis shows that there are certain differences in the studied properties and that the better results, with the treatment application, are achieved in the number of fruits per plant and yield per plant, even though they are not statistically confirmed. The pepper plants from the treatment, except that they achieved a larger fruit number and yield per plant, they were healthy, with no occurrence of pathogens which indirectly affect the level of the final yield, and also the product that is classified in organic food. That is a priority

from the human nutrition aspect, especially because the pepper, as most vegetable products, is commonly used in a fresh condition.

Conclusions

The research shown in this work is related to finding new technologies in pepper production, in a protected area, with alternative method application, in order to attain a better result in the number of fruits and the level of the yield per plant. With selected alternative methods, the plastic covered greenhouse pepper production would be advanced, which would avoid the use of chemical and synthetic preparations in the protection of possible pathogen attack. That is a preventive way that produces organic food. The average number of fruits per plant was 6.82. The average number was higher in the variant of microbiological application and *Trichoderma atroviride* (7.18 and 6.84). The yield per plant was higher in plants which were treated with microbiological fertilizer and Trichoderma for 14.24%, that is, 5.92%, while on the control variant, the yield of 664.6 g was measured. The recommendation in the plastic covered greenhouse production is to apply EM and to continue with the research of alternative preparations application in plant cultivation, especially in vegetable growing.

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INSECT PESTS OF STORED CEREAL IN NORTHERN CYPRUS

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Abstract

This study was carried out in order to determine insect pests of stored cereal in Northern Cyprus in the years of 2013-2014. Cereal samples were collected from total eight storages. The results obtained from this study indicated that *Anthicus floralis* Linnaeus (Coleoptera: Anthicidae), *Sitophilus granarius* Linnaeus (Coleoptera: Curculionidae), *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), *Cryptolestes (Laemophloeus) ferrugineus* Steph. (Coleoptera: Laemophloeidae), *Latheticus oryzae* Waterhouse (Coleoptera: Tenebrionidae), *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae), *Trogoderma* spp. (Coleoptera: Dermestidae), *Rhyzopertha dominica* Fabricius (Coleoptera: Bostrichidae), *Tenebrio* sp. (Coleoptera: Tenebrionidae), *Oryzaephilus surinamensis* Linnaeus (Coleoptera: Silvanidae), *Carpophilus* sp. (Coleoptera: Nitidulidae) and *Ptinus fur* Linnaeus (Coleoptera: Anobiidae) were determined as insect pests of stored cereal.

Key words: Cereal, storage, insect pests, Northern Cyprus.

Introduction

Cereal production is performed on an area of 84163 hectares within agricultural lands in Northern Cyprus and 92.7% of this production is barley, wheat is 6.4% and oat and triticale is 0.9% (Anonymous, 2011). Amount of products obtained from these lands is approximately 112.400 tons and additionally 56.318 tons of barley, corn, and wheat were imported for fodder (Anonymous, 2011) and kept in warehouses and stores for animals to feed. For this reason, production of cereals and especially barley has great importance for Northern Cyprus Farmers. These products are stored after harvest for consuming and stayed in storehouses for long times based on need, and get harmed by attacks of many pests in storage period. Stored products of agricultural and animal origin are attacked by more than 600 species of beetle pests, 70 species of moths and about 355 species of mites causing quantitative and qualitative losses (Rajendran, 2002; Rajendran and Sriranjini, 2008). Insects directly or indirectly cause loss by feeding on stored product. As a result of their feeding, they lead to weight loss of product, loss of seed properties, and negativities

in quality and nutritional values. They also cause significant decreases in quality features of the products due to their body residues, web and like substances they secreted. It adversely affects the human health in case that molding and rotting occur easily and intensely as the pest contamination is intense (Atabay et al., 2013). Agricultural products need to be protected with minimal levels of loss from harvest to consumption.

Losses generally caused by organisms of animal origin in stored products are accepted as averagely 10% per year (Donahaye and Messer, 1992). In developing countries, damage is between 10 to 40% (Shaaya et al., 1997). In some rural areas of Iran that uses traditional storages, damage caused by stored product insects can be as high as 80% (Modarres-Najafabadi et al., 2006). These products are more intensely exposed to attacks of numerous pest organisms due to unsuitable physical structures of the storehouses and the harm may increase more with the contaminations. Insects among these pests have a significant place. In grain storehouses, pests such as *Sitophilus* spp., *Tribolium* spp., *Rhizopertha dominica* F., *Attagenus piceus* Olivier, *Trogoderma granarium* Evert., *Oryzaephilus surinamensis* L. and *Ephestia kuehniella* Zell. cause losses of weight, germination, and quality during storage of grains (Erakay, 1974; Özar and Yücel 1982; Yücel, 1988; Özer et al., 1989; Anonymous, 1995; Işıkber et al., 2005).

There has been no study regarding insects causing harm in grain storehouses of Northern Cyprus up to now. This study was conducted in the years 2013 and 2014 in order to determine the insect species, harmful to stored products in Northern Cyprus where cereals are important and to reveal their significance.

Material and Methods

Studies were conducted in the months of November, March and May in 2013 and 2014 at totally 8 grain storehouses with capacity of 4.000-15.000 tons, including 2 in Akdoğan (Lefkoşa¹), 1 in Haspolat (Lefkoşa²) subject to Lefkoşa; 2 in Güzelyurt, and 3 in Mağusa (2 in Türkmenköy and 1 in İnönü). Sampling was made as aggregation from trashes in storage departments of each storehouse and from 5 different locations and different depths of stored product. Approximately 1 kg of sample was randomly taken from 5 to 10 gunny sacks of barley products stored in sacks and then aggregated. These samples were taken from aggregations prepared and put into fabric bags with the labels including information regarding the storehouse or storage information, and then brought to laboratory. Mature ones were determined by sieving the samples in bags, and their numbers were recorded after they were killed in killing bottles. Then, each sample was put into glass jars and covered with tulle cloth. Samples were kept at 25±1°C and 60-70% relative humidity. Number of hatching mature insects was added to resultant number for each sample by checking once a week. Observation was continued until hatching of mature was ended. Identification of these species was made by Prof. Dr. Abuzer YÜCEL.

Results and discussion

The study is conducted in grain storehouses and storages of Northern Cyprus in 2012 and 2013. It was determined that there are 9 families, 3 genera, and 8 species from Coleoptera order and 2 families and 2 species from Lepidoptera order (Table 1). Quantitative distribution of specified insect species was given based on years according to the storehouses where they were obtained (Table 2).

SECTION 3. PLANT PROTECTION - PHYTOMEDICINE

Table 1. Insects species identified in the grain warehouse of Northern Cyprus in 2013-2014 years

Order	Family	Common Name	Species
Coleoptera	<u>Anthicidae</u>	Narrownecked grain beetle	<i>Anthicus floralis</i> Linnaeus
	Curculionidae	Granary weevil	<i>Sitophilus granarius</i> Linnaeus
		Maize weevil	<i>Sitophilus zeamais</i> Motschulsky
	Laemophloeidae	Flat grain beetle	<i>Cryptolastes</i> (<i>Laemohploeus</i>) <i>ferrugineus</i> Steph.
	Tenebrionidae	Long-headed flour beetle	<i>Latheticus oryzae</i> Waterhouse
		Flour beetle	<i>Tenebrio</i> sp.
	Bostrichidae	Lesser grain borer	<i>Rhyzopertha dominica</i> Fabricius
	Silvanidae	Saw-toothed grain beetle	<i>Oryzaephilus</i> <i>surinamensis</i> Linnaeus
	Anobiidae	Whitemarked spider beetle	<i>Ptinus fur</i> Linnaeus
	Dermestidae	Khapra beetle	<i>Trogoderma</i> spp.
Lepidoptera	Nitidulidae	Sap beetles	<i>Carpophilus</i> sp.
	Pyralidae	Mediterranean flour moth	<i>Ephestia kuehniella</i> Zeller
	Gelechiidae	Angoumois grain moth	<i>Sitotroga cerealella</i> Olivier

Table 2. Number of pest species identified in the grain warehouse of Northern Cyprus 2013-2014 years and quantitative distribution

Species	Place and Years								Total
	Lefkoşa ¹ (Nicosia)		Lefkoşa ² (Nicosia)		Güzelyurt (Morphou)		Gazimağusa (Morphou)		
	2013	2014	2013	2014	2013	2014	2013	2014	
<i>Anthicus floralis</i>	-	-	-	-	17	-	-	13	30
<i>Sitophilus granarius</i>	1	-	39	211	15	5	1	58	330
<i>Sitophilus zeamais</i>	18	4	308	345	72	4	11	446	1208
<i>Cryptolastes</i> (<i>Laemohploeus</i>) <i>ferrugineus</i>	-	-	1	0	-	8	11	4	24
<i>Latheticus oryzae</i>	29	32	13	24	113	7	7	55	280
<i>Rhyzopertha dominica</i>	42	8	328	179	2907	18	7	211	3700
<i>Trogoderma</i> spp.	11	164	-	-	-	110	96	-	381
<i>Tenebrio</i> sp.	-	-	-	1	-	-	-	-	1
<i>Oryzaephilus</i> <i>surinamensis</i>	-	-	11	-	26	6	7	1	51
<i>Ptinus</i> <u>fur</u>	-	-	2	2	-	-	-	-	4
<i>Carpophilus</i> sp	-	-	-	2	-	-	-	-	2
<i>Ephestia kuehniella</i>	3	2	-	1	6	2	-	5	19
<i>Sitotroga cerealella</i>	12	-	-	-	5	-	-	-	17
Total	126	210	702	765	3161	160	140	793	6047

When Table 1 was examined, totally 10 species, including *Anthicus floralis* Linnaeus (Anthicidae), *Sitophilus granarius* Linnaeus, *Sitophilus zeamais* Motschulsky (Curculionidae), *Cryptolastes* (*Laemohploeus*) *ferrugineus* Steph. (Laemophloeidae), *Latheticus oryzae* Waterhouse (Tenebrionidae), *Rhyzopertha dominica* Fabricius (Bostrichidae), *Oryzaephilus surinamensis* Linnaeus (Silvanidae), *Ptinus fur* Linnaeus (Anobiidae) from Coleoptera order and, *Ephestia kuehniella* Zeller (Pyralidae) and *Sitotroga cerealella* Olivier (Gelechiidae) from Lepidoptera order, and also 3 genera including *Trogoderma* spp.(Dermestidae), *Tenebrio* sp. (Tenebrionidae), *Carpophilus* sp.

(Nitidulidae) from Coleoptera order at the genus level were determined. While the number of *Rhyzopertha dominica*, one of remarkable pests in the study was obtained at the lowest rate in samples taken from Güzelyurt in the year 2014, 2907 mature individuals were obtained from samples taken in the year 2013 (Table 2). The reason was that the samples were taken from sacks and aggregations of barley kept in storages for long times. It was also observed that all storehouses where the study was conducted were contaminated with this pest. This species can easily reach to uncontaminated storehouses since it has the ability to adapt dry conditions (Emekçi, *et al.*, 2004), and generally flies well (Stejskal *et al.*, 2003; Khan and Marwat, 2004). *Tenebrio* sp., *Carpophilus* sp. and *P. fur* were barely found among trashe compared to the other species.

Trogoderma granarium, *S. granarius*, *Sitophilus oryzae*, *Tribolium confusum*, *Tribolium castaneum*, *Palorus subdepressus*, *O. surinamensis*, *Attagenus piceus* Oliv., *Anthrenus verbasci* L., *R. dominica*, *Latheticus oryzae* Waterh., *E. kuehniella*, *Tenebroides mauritanicus* L., *Plodia interpunctella*, *Pyrallis farinalis* L., *Typhae stercorea* L., *Laemophleous* spp., *Tenebrioides mauritanicus*, *Liposoelis* spp., *Crytolestes* sp., *Dermestes* spp., *Ptinus* spp., *Scenopinus* spp. *Ahasverus advena* Walth, *Typhaea stercorea* L. species of insects were determined in the studies carried out at grain storehouses (Özar and Yücel, 1982; Anonymous, 1986; Aydın and Erdoğan, 1996; Işıkber *et al.*, 2005; Kucerova *et al.*, 2005; Belda and Riudavets, 2010). In both years, totally 6047 insects were obtained from 8 grain storehouses where the studies were conducted. It was observed that while storehouse with the highest level of contamination determined in surveys carried out on 3rd week of the November 2013 was located in Güzelyurt with 3161 insects, the storehouse with the lowest level determined in surveys carried out on 3rd day of the March 2014 was located in Akdoğan with 126 insects. As distribution of obtained species in the population was examined; it was determined that *R. dominica* was the most common species with the rate of 61.2%, which was followed by *S. zeamais* 20%, *Trogoderma* spp. 6.3%, *S. granarius* 5.5%, *L. oryzae* 4.6%, *O. surinamensis* 0.8%, *A. floralis* 0.5%, *C. (Laemohploeus) ferrugineus* 0.4%, *E. kuehniella* and *S. cerealella* 0.3%, *P. fur* 0.06%, *Carpophilus* sp. and *Tenebrio* sp. 0.03%, respectively (Figure 1).

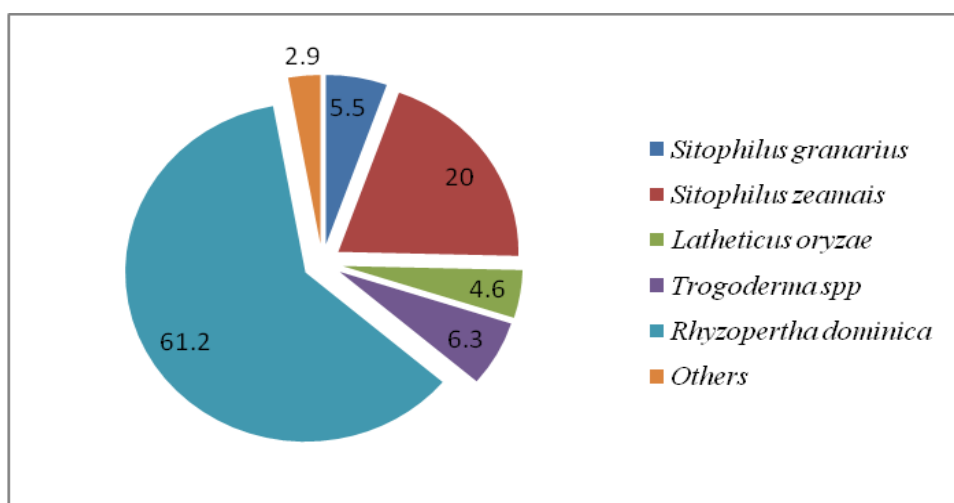


Figure 1. Distribution within the population of the species (%)

It was observed that as is stated at Table 2, 2 among these species had insignificant harm, 4 were secondary pests, and 7 were primary pests (CGC, 2015).

Table3. Economic importance of insects found in grain warehouse of Northern Cyprus

Species	Economic importance
<i>Anthicus floralis</i>	Minor pests
<i>Ptinus fur</i>	
<i>Latheticus oryzae</i>	
<i>Oryzaephilus surinamensis</i>	major (secondary) pests
<i>Tenebrio</i> sp.	
<i>Ephestia kuehniella</i>	
<i>Sitophilus granarius</i>	
<i>Sitophilus zeamais</i>	
<i>Cryptolastes (Laemohploeus) ferrugineus</i>	
<i>Sitotroga cerealella</i>	major (primary) pests
<i>Trogoderma</i> spp	
<i>Rhyzopertha dominica</i>	
<i>Carpophilus</i> sp.	

Sitophilus granarius, *S. oryzae*, *Rhyzopertha dominica*, *Trogoderma* spp., *Carpophilus* sp., *Cryptolastes (Laemohploeus) ferrugineus* and *Sitotroga cerealella* species were determined as the primary pest species harmful to solid grains. The Lesser grain borer, *R. dominica* was densely found as the most common among these species. This pest is quite significant because, it is commonly found in all storehouses and primary pest. Mature individuals and larvae of *R. dominica* cause serious harms in product by feeding on solid or broken grains, contaminating the product with wastes, and leaving scent on, and eliminating seed characteristics of wheat as a result of feeding on its embryo and endosperm (Campbell and Sinha, 1976; Edde, 2012). Mature individuals of the lesser grain borer were reported to cause weight loss of stored grains eight times more than larvae and their harm reached economic value 122 days after the contamination (6.4%) (Malagon and Trochez, 1985). The Rice weevil, *Sitophilus oryzae* and the Granary weevil, *S. granarius* species are in the second rank among primary pests intensively and commonly found in storehouses. These two species are very common in grain storages and cause significant quality and product losses by being directly fed in important kernels and contaminating the product (Kucerova *et al.*, 2003; Park *et al.*, 2003). *Trogoderma* species is at 3rd rank in terms of density. Being one of the most significant storage pests in the world, the Khapra beetle is an invasive species (Lowe *et al.* 2000). This insect, which is subjected to quarantine, is required to be eradicated from where it contaminated (Kerr 1981). *Trogoderma granarium* Everst and *T.inclusum* LeConte species are known to exist in Cyprus (<http://cyarthros.myspecies.info/content/checklist>). Density of *Cryptolastes ferrugineus*, *Ephestia kuehniella*, *Sitotroga cerealella* ve *Carpophilus* sp. species was found to be less. Primary pests in grain storehouses cause economic loss in products. Among the secondary pests, *Latheticus oryzae* and *Tenebrio* sp. species and *Oryzaephilus surinamensis* from Coleoptera order as well as *Ephestia kuehniella* species from Lepidoptera order were determined. These pests cause weight loss by being densely fed on products they are contaminated. The products are exposed to loss of quality because of the slough residues, wastes, and secreted web substances of pests. Molding, heat, and

putrification occur in product in case of dense contamination (Champ and Dyte 1976; Anonymous, 1995; Lorini, 2005). *Ptinus fur* and *A. floralis* species from Coleoptera order were also determined in grain storehouses. However, the damage caused by these insects was reported to be insignificant (CGC, 2015).

Conclusions

Thirteen insect species were determined in grain storehouses of Northern Cyprus as a result of the study. It was recorded that among them, 7 species were primary significant, 4 species were secondary significant, and 2 species were insignificant (Table 3). *Rhyzopertha dominica* was the most common and dense, which was followed by *Sitophilus*, and *Trogoderma* species, and *Latheticus oryzae* species. Mentioned species were found in grain storehouses of Lefkoşa, Güzelyurt and Gazimağosa. It was observed that physical structures of most of the grain storehouses, from which the samples were taken, were not suitable for storing products and the pest management failed to satisfy. Consequently, it was concluded that the improvement of basic matters such as enhancement of storage conditions and physical structures of storehouses, and their cleaning, as well as taking serious precautions, in the shortest time against the pests subjected to internal and external quarantine such as *T. granarium* would be useful.

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UDC: 633.71-153.037:632.48]:632.952

632.952:[633.71-153.037:632.48

Original scientific paper

THE IMPACT OF STROBILURIN FUNGICIDES IN PREVENTION OF DAMPING OFF DISEASE ON TOBACCO SEEDLINGS

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Abstract

Damping off disease, caused by soil pathogens *Rhizoctonia solani* and *Pythium debaryanum*, is very frequent in tobacco seedbeds. Various fungicides are applied to prevent the spread of infection in seedlings. In our research, tests were made with strobilurin fungicides in order to estimate their effectiveness in control of the disease causing agents. The tests were carried out during the 2014 in the biological laboratory of the Scientific Tobacco Institute – Prilep. Seedlings of the variety NS72 were sown in 0.3 m² plastic trays. Before sowing, inoculum prepared from culture of the pathogenic fungi grown on nutrient media PDA was added to the soil. The test was performed with following fungicides: Signum 0.1% (boscalid 267 g/kg + pyraclostrobin 67 g/kg), Quadris 25SC 0.075% (azoxystrobin 250 g/l) and Stroby WG 0.02% (kresoxim-methyl 500g/kg). The check variant was consisted of untreated plants. Two watering's were applied on seedlings with fungicide suspensions (1 L/m²). The first watering was applied prior to rapid growth stage and the second one 15 days after. The coefficient of fungicide effectiveness was calculated by applying Abbott's formula. In the seedlings treated with fungicides no signs of disease were observed, while in the check variant the infection rate was 90-100%. Although grown in artificially inoculated soil with the culture of pathogenic fungi, the seedlings treated with fungicides were healthy and in good condition. Because of the high effect achieved with the chemicals, they can find wider practical application in protection of tobacco seedlings from the above soil pathogens and in production of healthy and high quality planting material.

Key words: tobacco seedlings, pathogens, inoculum, fungicides, effectiveness.

Introduction

Damping off, caused by soil pathogenic fungi *Rhizoctonia solani* and *Pythium debaryanum*, is very common disease in tobacco seedlings. The favorable microclimate conditions in seedbeds contribute to its mass occurrence, resulting in serious seedling loss. The production of healthy and high quality seedlings for transplanting is an important condition for growing healthy tobacco crop. To prevent the spread of the disease in seedlings, various practices are used, including mechanical, agro-technical, biological and chemical methods of protection. Great attention has been paid to the strobilurin group of fungicides. They include many synthetic plant-protective compounds with broad spectrum

of activity (Herms et al., 2002). The authors reported that F500 fungicide (pyraclostrobin) improves the resistance of tobacco to TMV and *Pseudomonas syringae* pv. *tabaci*. Plants treated with strobilurins are in good health, better developed and have a more intensive green color, compared to untreated plants. It is considered that salicylic acid induces resistance to all plant pathogens including bacteria, fungi and viruses, but the mechanism by which the acid develops resistance to viruses is not known (Chivasa et al. 1997). Venancio et al. (2003) also showed that salicylic acid induces resistance to Tobacco mosaic virus (TMV). According to Kanungo and Joshi (2014), plants treated with strobilurin products have distinctive dark green color, larger biomass and higher yield. Venancio et al. (2003) confirmed that two weeks after treatment the biomass increased up to 20%. In 1999, Grossmann et al. (cit. Swoboda and Pedersen, 2005) identified some physiological changes in tobacco and wheat plants treated with strobilurin. According to Nason et al. (2007) and Kanungo and Joshi (2014), however, various strobilurin fungicides have the same mode of action and produce similar effects on plant physiology.

The aim of this investigation was to study the effectiveness of several strobilurin fungicides in our conditions for protection of tobacco seedlings from *R. solani* and *P. debaryanum* – phytopathogenic fungi that cause the damping off disease.

Material and methods

Investigation was carried out during the 2014, in the biological laboratory of Tobacco Institute - Prilep. Seedlings of NS72 variety were planted on 7.5.2014 in 0.3 m² plastic trays, in soil previously inoculated with pure fungal culture. The trial was set up in three replications. Fungal cultures were grown in potato-dextrose agar in a thermostat at 25° C for 10 days. The inoculum for one tray was prepared from mycelial colony in two Petri dishes, blended in 200 ml of distilled water and added to the soil before sowing the seedlings. Two treatments with fungicides were made during seedlings growth, the first one in the stage of 4th leaf on 21.5.2014 and the second 15 days later, on 5.6.2014. The seedlings were treated with 1 L fungicide solution/m² and check variants were poured only with pure water.

Health condition of tobacco seedlings was assessed by the number of infected plants and by the percentage of plant area that was affected. After the second treatment two assessments were made at an interval of 10 to 15 days. After the inspection of health condition of seedlings, the infected area was measured in the sites where infection occurred. The measurement data were used to calculate the percentage of empty area, i.e. disease intensity. According to the disease intensity in the second assessment, coefficient of fungicides effectiveness was calculated by the Abbott's formula.

Table 1. Investigated fungicides

Fungicide	Active ingredient	Concentration %
Proplant 722 SL	722 g/l propamocarb hydrochlorid	0,25
Top M	70% thiophanate methyl	0,1
Signum	267 g/kg boscalid + 67 g/kg pyraclostrobin	0,1
Quadris 25SC	250 g/l azoxystrobin	0,1
StrobyWG	500g/kg kresoxim-methyl	0,1

Results and discussion

Effectiveness of the investigated fungicides in protection of tobacco seedlings from *P. debaryanum* is presented in Table 2. The disease appeared with different intensity. The highest intensity was recorded in the check variant (Photo 1), ranging from 80.00% in the first assessment to 90.00% in the second.

Table 2. The intensity of disease in seedlings grown in soil infected with inoculum of *P. debaryanum*

Variant	Infected area %		Effectiveness %
	I Assessment	II Assessment	
Check	80,00	90,00	-
Proplant 0,25%	30,00	34,67	61,47
Signum 0,1%	0,00	0,00	100,00
Quadris 0,1%	0,00	0,00	100,00
Stroby 0,1%	0,00	0,00	100,00

According to the above data, the infection of seedlings treated with the standard fungicide Proplant 0.25% ranged 30.00% in the first assessment and 34.67% in the second. The effectiveness of seedlings protection with this fungicide reached 61.47%. 100% effectiveness was achieved in variants treated with Signum 0.1%, Quadris 0.1% and Stroby 0.1%. Seedlings were in good health condition and with intensive green color (Photo 2). Herms et al. (2002) and Kanungo and Joshi (2014) in their investigations confirmed that plants treated with pyraclostrobin are of intensive green color and have larger biomass compared to the untreated plants. Significant differences in the incidence of damping off disease on tobacco seedlings caused by *P. debaryanum* were observed in plants treated with fungicides based on azoxystrobin and etridiazole (Gutierrez, 2012), with the highest percentage of healthy plants and low percentage of infection.



Photo 1. *P. debaryanum* - check



Photo 2. Seedlings treated with Signum

Similar results were obtained in seedlings cultivated in soil inoculated with the pathogenic fungus *R. solani*. In the first assessment, the infected area in the check variant was 83.33%. In the second assessment the disease intensity reached 100% and the seedlings were almost completely destroyed (Table 3).

Table 3. Intensity of the disease in seedlings grown in soil infested with inoculum of *R. solani*

Variant	Infected area		Effectiveness %
	I Assessment	II Assessment	
Check	83,33	100,00	-
Top M 0,1%	45,00	45,00	55,00
Signum 0,1%	0,00	0,00	100,00
Quadris 25SC 0,1%	0,00	0,00	100,00
StrobyWG 0,1%	0,00	0,00	100,00

The effectiveness achieved by application of the standard fungicide Top M 0.1% was 55.00% and the disease intensity was 45.00%. The effectiveness achieved with strobilurin fungicides Signum 0.1%, Quadris 0.1% and Stroby 0.1%, was 100%, due to which no occurrence of infected plants was recorded (Photo 3, 4, 5).



Photo 3. Seedlings treated with Signum



Photo 4. Seedlings treated with Quadris



Photo 5. Seedlings treated with Stroby

La Mondia (2012) also confirmed that Quadris (azoxystrobin) greatly reduces the intensity of disease caused by *R. solani* in tobacco seedlings compared to the untreated check and can significantly reduce the fungal inoculum in the soil. This confirms that fungicides from the strobilurin group can provide high protection of tobacco seedlings from the investigated soil pathogens.

Conclusion

Investigated fungicides showed high effectiveness in protection of tobacco seedlings from the causing agents of damping off disease. The high effectiveness was achieved at an extremely high intensity of attack, which in the check variants reached 90% in *P. debaryanum* and 100% in *R. solani*.

By application of the standard fungicides Proplant in concentration of 0.25% the achieved effectiveness was 61.47% in *P. debaryanum*. With Top M in concentration of 0.1%, the achieved effectiveness was 55.00% in *R. solani*.

The highest effectiveness in the control of both pathogens was achieved by application of strobilurin fungicides and variants treated with these chemicals showed no symptoms of disease. It is of particular importance that the seedlings treated with strobilurin are healthy, with intensive green color and better development.

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UDC: 632.782:633.71(497.775)"2010/2012"

633.71-278.2(497.775) "2010/2012"

Original scientific paper

***PHTHORIMAEA OPERCULELLA* (ZELLER), PEST CONTROL ON TOBACCO**

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Abstract

Phthorimaea operculella is an oligophagous pest, whose larvae feed on Solanaceae family. We observed *P. operculella* as pest in commercial tobacco fields in three locations in Varos -Prilep (2010-2012) during the entire tobacco vegetation. Investigations were carried on 100 tobacco stalks per location. Four insecticides were tested against larvae, in field and laboratory conditions. These insecticidal chemical compounds belong to different groups: chlorpyrifos+cypermethrin, deltamethrin, methomyl, chlorpyrifos. *P. operculella* is a small brown to light grey moth (approximately 9 mm long with a 14 mm wingspan). The young larva is flexible and active. The neonate body is light brown, and shifts to green or pink in later instars. After hatching the larvae are 1.2 to 2 mm long. The larva grows to a length of about 1-1.4 cm. Monitoring of *P. operculella* is one of the most important components of an integrated pest management (IPM). Larvae create tunnels in tobacco leaves, feeding on parenchyma and leaving only the upper and lower epidermis. The tunnel can reach about 11 cm in size. The damaged leaf tissue dies and leaves the leaf with a split appearance, reducing quality and therefore, causing economic loss. In Varos, in 2012 we observed increased damage caused by *P. operculella* on tobacco (35.28% attacked tobacco stalks). As in-field population increases, ten or more mines can develop on an individual tobacco plant. Larvicidal effect of deltamethrin and chlorpyrifos+cypermethrin yielded 100% mortality of caterpillars from the second age group in laboratory conditions, followed by chlorpyrifos (96.7%) and methomyl (90.0%). Larvae spend their entire lifetime in tunnels and with the removal of lower leaves will stop the larvae development inside leaf mines and thereby reduce their field populations. Other IPM recommendations are: determine of damage threshold, use of pheromone traps, planting only healthy tobacco stalks, biological control, autumn plowing etc.

Key words: tobacco, *Phthorimaea operculella*, pest control.

Introduction

Phthorimaea operculella (Zeller, 1873) belongs to order Lepidoptera, suborder Ditrysia, family Gelechiidae, subfamily Gelechiinae, tribe Laspeyresiini, genus *Phthorimaea* Shull, 1909, species *operculella* Zeller, 1873. Synonyms: *Gelechia operculella* Zeller, 1873,

Gnorimoschema operculella Zeller, *Bryotropha solanella* Boisduval, *Gelechia terrella* Walker, 1864, *Gelechia sedata* Butler, 1880 ect (8, 13).

P. operculella is spread worldwide, because of its high adaptability to daily and seasonal changes and high reproductive potential. Larva bores into the leaves, shoots and stems of the plants, as well as root tubers, of the Solanaceae family: potato, tomato, tobacco, eggplant and non-crop hosts. It is potentially serious pest of tobacco. On tobacco it is known as tobacco leaf miner or tobacco splitworm (7, 13).

Early mines on tobacco leaves can be easily mistaken for abiotic damage or chemical burn. Mines are more visible by the 3rd larval instar. Larvae create mines, removing photosynthetic tissue and leaving only the upper and lower epidermis, resulting in a “window-pane” appearance (6, 7).

Material and methods

We observed *P. operculella* as pest in commercial tobacco fields in three locations in Varos near Prilep (2010-2012) during the entire tobacco vegetation. Investigations were carried on 100 tobacco stalks per location, in a period of 7 days or 400 plants per month. During investigations, each plant was observed for mines and mine location (middle, lower, or upper third of the plant).

Field collected material was analysed, nourished and bred in the laboratories of Scientific Tobacco Institute. Four insecticidal chemical compounds were tested against tobacco splitworm larvae (chlorpyrifos + cypermethrin, deltamethrin, methomyl, chlorpyrifos). The insecticides were applied foliary, in field with knapsack sprayer, at 20°C. In control plots, fresh tap water was sprayed on the crop. In laboratory trial, 30 larvae of the tobacco spit worm for each variant were further bred in petri dishes according standard laboratory methodology. During larval stages larvae were nourished with treated tobacco leaves.

Results and discussion

P. operculella is a small brown to light grey moth (approximately 9 mm long with a 14 mm wingspan). Wings of moth are roof-like. Fore-wings are yellowish-brown grey, widely lanceolate. A longitudinal, blackish stripe and dark points form along median crease. Hind-wings are almost as wide as fore-wings, with folded external edge and a fringe longer than it is wide. Hind wings are grey, and bear long bristles (Photo1). The young larva is flexible and active. The neonate body is light brown, and shifts to green or pink in later instars (Photo 2). After hatching the larvae are 1.2 to 2 mm long. The larva grows to a length of about 1-1.4 cm (Photo 3).



Photo 1. *P. operculella*



Photo 2. Larvae of *P. operculella*



Photo 3. Larvae of *P. operculella*



Photo 4. Damages of *P. operculella*



Photo 5. Damages of *P. operculella*

Larvae mine and split the tobacco leaves and the damaged leaf tissue dies. Mines look like long blisters or transparent leaf windows. Sometimes larvae bore into stalk tissue and leaf mid-ribs (Photo 4). Larvae spend their entire lifetime in tunnels. The tunnel can reach about 11 cm in size.

Pupae are 0,6- 0.8 cm long, turn golden brown and deepen to brown-black before eclosion (Photo 6)

Photo 6. Pupa of *P. operculella*

Duration of different stages of *P. operculella* reared on tobacco in laboratory conditions is presented in Table 1.

Table 1. Duration of different stages of *P. operculella* reared on tobacco

Developmental Stages	<i>P. operculella</i>
Egg	3
Total larval period	15-20
Pupa	5-7
Pre –oviposition period	2-3
Oviposition period	4-6

Monitoring of *P. operculella* is one of the most important components of an integrated pest management (IPM).

P. operculella infestation in tobacco starts on the lower leaves of tobacco plant, because they are closer to the oviposition site. As in-field population increases, ten or more mines can develop on an individual tobacco plant. Larvae have been observed on upper stalk leaves in 2011 and 2012 (Tab. 3 and 4).

During tobacco vegetation *P. operculella* occurs with a different quantitative representation, depending on the climatic conditions. *P. operculella* prefers drier summers with higher temperatures. In Varos we observed increased damage caused by *P. operculella* on tobacco, especially in 2011 and 2012.

Climate conditions in 2010 were less favorable for occurrence of tobacco leaf miner. (with total precipitation of 298.0 mm, 35 days with rainfall, and mean monthly air temperature of 18,9 °C) We obtained 307 tobacco stalks attacked by *P. operculella* in 2010 (Table 2).

Table 2 Number of tobacco stalks attacked by *P. operculella*-2010

Tobacco plots	July	August	September	Total
I	99	17	10	126
II	84	11	7	102
III	61	6	2	69
Total	244	34	29	307

In 2011 *P. operculella* caused a more severe damage to tobacco fields in Varos and we found 828 tobacco stalks attacked by *P. operculella* (Table 3). The mean monthly air

temperature is 19.1°C, total precipitation is 180.0 mm, and by the end of June and in July and August the precipitation amount was very low.

Table 3 Number of tobacco stalks attacked by *P. operculella*-2011

Tobacco plots	July	August	September	Total
I	184	114	65	363
II	68	92	29	189
III	105	98	73	276
Total	357	304	167	828

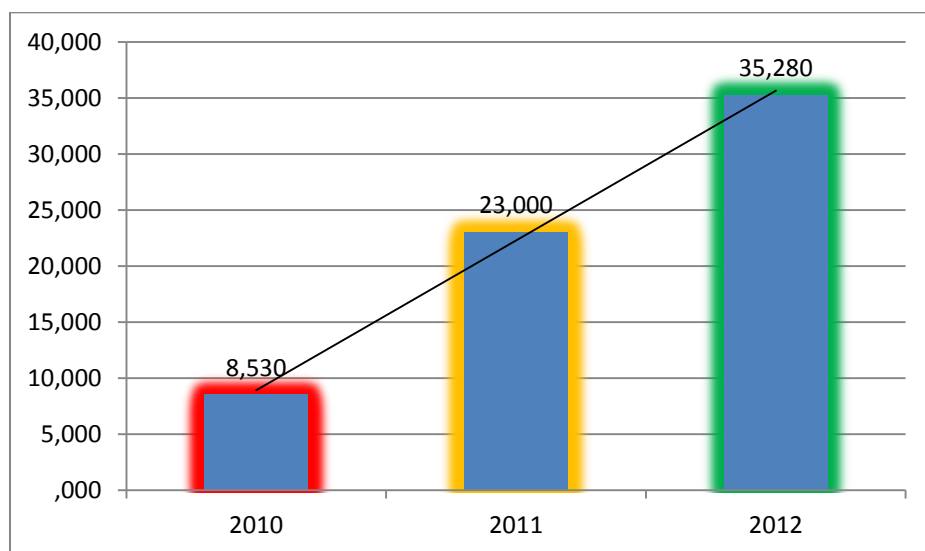
Favorable conditions in 2012 enabled a more abundant occurrence of *P. operculella* on tobacco during the whole growing season and we found 1270 damaged tobacco stalks by *P. operculella* (Table 4). In 2012 the mean monthly air temperature is 20.3°C, total precipitation is 186.0 mm, with one day with precipitation in June and two in July, which made favorable conditions for occurrence of this pest.

Table 4 Number of tobacco stalks attacked by *P. operculella*-2012

Tobacco plots	July	August	September	Total
I	191	182	65	438
II	148	131	77	356
III	205	175	96	476
Total	544	488	238	1270

The difference in infestation level can be influenced by other factors such as microclimate, plant health, irrigation, and weed or alternate host presence.

In Varos, in 2012 we observed increased damage caused by *P. operculella* on tobacco with total 35.28% attacked tobacco stalks (Graph. 1).



Graph. 1 Percentage representation of damage tobacco stalks with *P. operculella*

Larval densities on tobacco plants were significantly higher at the margins of the fields than in the centre in 2010 and 2011 (Table 5). Data obtained in this study are in agreement with those obtained on potatoes (1).

Table. 5. Quantitative representation of tobacco splitworm larvae on tobacco

Replication	No. of tobacco stalks	Total No. of larvae	Place of sampling			
			Plot margins		Middle of the plot	
			No. of larvae	attacked plants %	No. of larvae	attacked plants %
2010	3600	307	215	70,00	92	30,00
2011	3600	828	512	61,84	316	38,16
2012	3600	1270	659	51,89	611	48,11

From Table 5 it can be concluded that the percentage of plants infested by larvae of the tobacco splitworm ranged from 30% in 2010, 38,16 % in 2011 and 48,11% in 2012 in the middle of the plots, to 70% in 2010, 61,84 % in 2011 and 51,89% in 2012 to the margin parts of the plots.

This harmful moth can be controlled by using a combination of cultural practices, including destruction of tobacco stalks from the field immediately after harvest and autumn plowing, which will reduce its overwintering sites.

Larvae need living plant material to survive and with moving of infested leaf material out of the field will cease *P. operculella* feeding and will kill larvae.

Results of effectiveness of some insecticides in the control of *P. operculella* larvae are presented in Table 6 and 7.

Table 6- Effectiveness of some insecticides in the control of *P. operculella* -2011

Chemical	Koncen.	No. of larvae	2011			
			Mortality of larvae		No. P	No. AE
			No. DL	%		
chlorpyrifos +cypermethrin	0,15%	30	29	96,7	1	0
chlorpyrifos	0,15%	30	28	93,3	2	1
deltamethrin	0,04%	30	29	96,7	1	0
methomyl	0,04%	30	27	90.0	3	2

Legend:DL -dead larvae, P-pupa, AE- Adults eclosion

Table 7- Effectiveness of some insecticides in the control of *P. operculella*-2012

Chemical	Koncen.	No. of larvae	2012			
			Mortality of larvae		No. P	No. AE
			No. DL	%		
chlorpyrifos +cypermethrin	0,15%	30	30	100	0	0
chlorpyrifos	0,15%	30	29	96,7	1	0
deltamethrin	0,04%	30	30	100	0	0
methomyl	0,04%	30	27	90.0	3	1

Legend:DL -dead larvae, P-pupa, AE- Adults eclosion

Larvicidal effect of deltamethrin and chlorpyrifos+cypermethrin yielded 100% mortality of caterpillars from the second age group in laboratory conditions (Table 7 and Photo 7), followed by chlorpyrifos (96.7%) and methomyl (90.0%). The best moment for treatment with an insecticide is while the larvae are very small, neonate larvae that had not yet penetrated the leaf. Then larvae spend their entire lifetime in tunnels, removing photosynthetic tissue and leaving only the upper and lower epidermis, resulting in a “window-pane” appearance.



Photo 7. Chemical control of larvae

Foliar infestation may be severe enough to destroy the plant. Also, fungi and mites develop inside the galleries causing decomposition of the leaves. The attacked tobacco is breakable and unsuitable for fabrication.

Tobacco fields with a known history of *P. operculella* infestation should be avoided in rotation with non- host crops when possible. Do not plant tobacco in fields or near fields which, during the previous crop, were heavily infested with *P. operculella* if it is possible.

Conclusions

In tobacco, larvae of *P. operculella* -tobacco splitworm primarily act as leaf-miners.

The damaged leaf tissue dies and leaves the leaf with a split appearance, reducing quality and therefore, causing economic loss.

Understanding of the morphology, biology and phenology of *P. operculella* populations infesting tobacco can be of great help in predicting potentially severe infestations.

All applied insecticides showed high effectiveness in control of *P. operculella* on tobacco.

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**EXTRACELLULAR ENZYME ACTIVITY OF *TRICHODERMA* STRAINS
ISOLATED FROM DIFFERENT SOIL TYPES**Danilović G.¹, Radić D.², Raičević V.², Jovanović Lj.³, Kredics L.⁴, Panković D.^{1*}¹Faculty of Environmental Protection, Educons University, Sremska Kamenica, Serbia²Faculty of Agriculture, University of Belgrade, Belgrade, Serbia³Faculty of Ecological Agriculture, Educons University, Sremska Kamenica, Serbia⁴Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

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Abstract

Enzymatic characterization of 16 *Trichoderma* strains, grown in potato dextrose broth was carried out semi-quantitatively by API-ZYM test. Strains that were used for test were isolated from different soil types and belonged to *T. harzianum* species complex, *T. koningiopsis*, *T. atroviride*, *T. brevicompactum*, *T. gamsii*, *T. citrinoviride* and *T. longibrachiatum*.

The activities of acid phosphatase and naphthol-AS-BI-phosphohydrolase were high in all examined strains and N-acetyl- β -glucosaminidase activity was detected in most of them. However, activities of β -glucuronidase, α -glucosidase, β -glucosidase were negative. Among all investigated *Trichoderma* isolates, *T. brevicompactum* showed moderate α -galactosidase and β -galactosidase enzymatic activity.

Obtained results are in accordance with previous results on very good antagonistic properties of examined *Trichoderma* strains against fungal pathogens, indicating that extracellular enzymes are important part of their antagonistic mechanism of biological control.

Key words: *Trichoderma*, N-acetyl- β -glucosaminidase, biocontrol, API-ZYM.

Introduction

Trichoderma spp. is cosmopolitan soil borne fungi with great abilities of colonizing their habitats either by efficient utilization of substrate or their secretion capacity for enzymes and antibiotics (Schuster and Schmoll, 2010). They play major role as biocontrol agents having capabilities of increasing crop yield acting as biopesticides, bioherbicides or plant growth promoters. Their antagonistic potential can be based on various mechanisms. *Trichoderma* strains exert biocontrol against fungal phytopathogens either indirectly, by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and plant defensive mechanisms with inducing plant systemic resistance to pathogens and antibiosis, or directly, by mechanisms such as mycoparasitism. The importance of these mechanisms is that they may act coordinately and their process of biocontrol depends on different factors. Activation of each mechanism follows the production of specific compounds and metabolites, such as plant growth factors, hydrolytic

enzymes, siderophores or antibiotics (Benítez *et al.*, 2004). Strains belonging to the genus *Trichoderma* are well known to produce a lot of different extracellular enzymes. The amount and activity of these enzymes are important in the biofungicide efficacy of the strains, as they can determine the antagonistic properties of the strains (Sharma *et al.*, 2012).

One of the mechanisms of biocontrol which is based on production of extracellular enzymes which are responsible for degradation of fungal cell walls is mycoparasitism. The chitinase, laminarinase and protease systems play important roles in the mycoparasitism of *Trichoderma* spp. (Sivan and Chet, 1989). N-acetyl- β -glucosaminidase forms part of the chitinase complex: two isoenzymes were described in the most isolated species, *T. harzianum* (Haran *et al.* 1995). Chitinase encoding genes are also the most used to improve plant defence against fungal pathogens (Sharma *et al.*, 2012). These enzymes are able of degrading the linear homopolymer of β -1,4-Nacetyl- D-glucosamine, the major component of the cell wall of most phytopathogenic fungi, as well as are very strong inhibitors of their hyphal growth. (Harman *et al.*, 2004).

Enzyme β -1,3-glucanase is known to inhibit germination of spores or the growth of pathogens and often work in correlation with chitinase and antibiotics. These enzymes in fungi have functions in morphogenetic processes during their development and differentiation. In the fasting state these enzymes become autolytical, providing carbon and energy and finally they are involved in mycoparasitism (El-Katatny *et al.*, 2001).

One of the methods used to detect extracellular enzyme activity is the API ZYM system. This semi quantitative method is designed to detect 19 enzymatic reactions. It was used as one of the methods for the investigation of the taxonomy of different microorganisms including fungi (Bridge and Hawksworth, 1995). In this paper we have used API ZYM system to investigate extracellular enzyme activity of *Trichoderma* strains with very good antagonistic properties (Danilovic, *et al.*, 2014) isolated from different soil types.

Material and methods

Trichoderma strains used in this study were isolated from different soil types in Serbia: Vertisol, Rendzic Leptosol, Chernozem, Regosol, Fluvisol, Calcic Gleysol, Eutric Cambisol and Dystric Leptosol. Sixteen strains that were used belonged to *T. harzianum* species complex (SZMC 20965; SZMC 20969; SZMC 20982; SZMC 20988; SZMC 20994), *T. koningiopsis* (SZMC 20983 and SZMC 20984), *T. atroviride* (SZMC 20968), *T. brevicompactum* (SZMC 22661 and SZMC 22663), *T. gamsii* (SZMC 20985), *T. citrinoviride* (SZMC 20987 and SZMC 22668) and *T. longibrachiatum* (SZMC 22664, SZMC 22669 and SZMC 22665). The strains were maintained on potato dextrose agar medium (PDA).

Detection of the enzymatic activity

Trichoderma strains were preincubated on PDA medium at 25°C for 5 days. Mycelia discs of 5 mm diameter were cut from the edge of petri dish and inoculated into 200 ml of sterile potato dextrose broth (PDB) liquid medium. Flasks were shaken for 7 days on 25°C in the dark on an orbital shaker BIOSAN (Lithuania). Liquid culture was filtrated through sterile gauze and filtrate was used for the detection of extracellular enzymatic activity by using

the semi quantitative API ZYM system (bioMerieux, France), according to the manufacturer protocol.

Sixty five micro liters of each inoculum was transferred into each of the twenty API ZYM strip microtubes prefilled with 5 ml of distilled water for maintaining humidity and incubated at 37°C in thermostat for 4h. After incubation period a drop of ZYM A and ZYM B reagents was added to each of twenty wells.

The color reaction was read after 5 minutes according to the API ZYM reading color scale. According to this scale enzyme activities were scored as following: 0-no color, 1-low, 2-moderate and 3-high.

Results and discussion

Results of the enzymatic activity are presented in Table 1. The activities of acid phosphatase and naphthol-AS-BI-phosphohydrolase were high in the case of almost all examined isolates. Our results show that out of 16 examined strains only 2 are classified with low acid phosphatase activity. Altomare *et al.* (1999) have shown that *Trichoderma* is highly competitive for P-uptake with some phytopathogenic fungi, which are therefore suppressed. This might be based on high activities of acid phosphatase and naphthol-AS-BI-phosphohydrolase. In plants acid phosphatase increase phosphate availability, leading to improved growth of plants in the presence of *Trichoderma* strains in the rhizosphere (Kapri and Tewari, 2010). The activity of N-acetyl- β -glucosaminidase, was detected in most examined strains. This enzyme is included in chitinolytic system of *Trichoderma* species and responsible for breakdown of the pathogen cell walls, i.e. involved in antagonism mechanisms. Also it is used for the production of liquid, powder or solid antifungal compositions for plant protection and was firstly isolated from *T.harzianum* P1 strain (Harman *et al.*, 1995). Activities of β -glucuronidase, α -glucosidase, β -glucosidase were negative in case of all examined samples. Among all investigated *Trichoderma* isolates, *T.brevicompactum* showed moderate α -galactosidase and β -galactosidase enzyme activity. It is reported that α -galactosidase can be used in modifications of diverse agricultural products containing raffinose-family oligosaccharides to digestible sugars (Shabalin *et al*, 2002). Thus it could be presumed that *T.brevicompactum* strain is a good source of α -galactosidase enzyme and it could help in the hydrolysis of galactooligosaccharides.

Table 1. Extracellular enzyme activity as determined with API-ZYM system. Numbers in columns represent different *Trichoderma* strains, as explained at the bottom of the table.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Alkaline phosphatase	1	1	0	0	1	0	1	1	0	0	0	3	1	0	1	1
Esterase (C4)	3	3	0	0	1	1	0	2	1	0	2	3	0	0	0	2
Esterase Lipase (C8)	3	3	0	0	1	1	1	1	0	0	1	1	0	0	0	1
Lipase (C14)	2	2	0	0	1	1	1	1	0	0	0	1	1	0	0	1
Leucine arylamidase	0	0	0	0	0	0	0	1	1	0	0	1	0	0	1	0
Valine arylamidase	0	0	0	0	0	1	0	1	0	0	0	1	0	0	1	0
Cysteine arylamidase	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0
Trypsin	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	1
α -chymotrypsin	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0
Acid phosphatase	3	3	2	3	3	3	2	3	2	3	2	3	1	1	3	3
Naphthol-AS-BI-phosphohydrolase	3	3	2	3	3	3	2	3	2	3	2	3	1	1	3	3
α -galactosidase	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1
β -galactosidase	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
β -glucuronidase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
α -glucosidase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
β -glucosidase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N-acetyl- β -glucosaminidase	1	1	1	3	2	3	0	0	2	3	3	3	0	1	2	0
α -mannosidase	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0
α -fucosidase	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
1- <i>T.brevicompactum</i> SZMC 22661; 2- <i>T.brevicompactum</i> SZMC 22663; 3- <i>T.longibrachiatum</i> SZMC 22664; 4- <i>T.citrinoviride</i> SZMC 22668; 5- <i>T.longibrachiatum</i> SZMC 22669; 6- <i>T.longibrachiatum</i> SZMC 22665; 7- <i>T.harzianum</i> SZMC 20994; 8- <i>T.harzianum</i> SZMC20988; 9- <i>T.harzianum</i> SZMC 20969; 10- <i>T.citrinoviride</i> SZMC 20987; 11- <i>T.harzianum</i> SZMC 20965; 12- <i>T.koningiopsis</i> SZMC 20983; 13- <i>T.gamsii</i> SZMC 20985; 14- <i>T.harzianum</i> SZMC 20982; 15- <i>T.atroviride</i> SZMC 20968; 16- <i>T.koningiopsis</i> SZMC 20984																

Previously communicated (Danilovic *et al.*, 2014) very good antagonistic properties of examined strains are connected with high activities of acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl- β -glucosaminidase. In case of *T.brevicompactum* strains, two more enzymes: α -galactosidase and β -galactosidase are contributing to their antagonistic potential.

Most of the examined *Trichoderma* strains are promising candidates for practical application within the frames of biological control. Our results indicate that extracellular enzymes are important part of their antagonistic mechanism against phytopathogens.

Acknowledgement

The research is co-financed by the Ministry of Education and Science of the Republic of Serbia (Project No. III43010 and TR 31080).

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UDC: 635.64-248.216.5:632.934]:631.544.1(497.7)

635.64-248.216.5:632.937.1]:631.544.1(497.7)

Original scientific paper

BIOLOGICAL AND CHEMICAL CONTROL OF GRAY MOLD DISEASE (*BOTRYTIS CINEREA*) OF TOMATO GROWN UNDER PLASTIC TUNNELS

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Abstract

The effect of biological treatments with Serenade[®]Max and chemical treatments with Signum[®] against the gray mold of tomato, caused by *Botrytis cinerea*, was evaluated during the 2012, in two regions in the Republic of Macedonia on plants grown under high tunnels. Stem canker, caused by *Botrytis cinerea* was dominant symptom on tomato plants in the region of Valandovo, while in the region of Strumica the symptoms of gray mold were prevalent on fruits. Serenade[®]Max (*Bacillus subtilis* QST 713) applied as preventive sprays provided excellent protection from gray mold of tomato in both regions. Serenade[®]Max reduced the disease incidence by an average of 87% (87,7% in the region of Valandovo and 87,5% in the region of Strumica). Fungicide Signum (boscalid+pyraclostrobin), applied as preventive sprays attained 93,8% and 97,5% reduction of gray mold disease in Valandovo and Strumica, respectively. The results showed that biofungicide Serenade[®]Max has a prospective use for control of gray mold disease in tomato.

Key words: tomato, gray mold, *Bacillus subtilis*, boscalid+pyraclostrobin.

Introduction

Tomato is one of the most significant vegetable crops that are cultivated in R. Macedonia. It is produced on over 5700 ha and it represents a leading vegetable culture in the Strumica region (Tudzarov, 1990). Most of the tomato production is cultivated in glass houses and plastic tunnels. This kind of intensive tomato production is favorable for development of various pathogen organisms (Kuzmanovska, 2011). Among the most spread tomato pathogens are *Pythium spp*, *Phytophthora infestans*, *Alternaria solani*, *Alternaria alternata*, *Botrytis cinerea* et al. (Jovanchev, 1997). *Botrytis cinerea* – the cause of gray mold disease is a polyphagous phytopathogen fungus (Giraud et al., 1997; Ma & Michailides, 2005; Zhao et al., 2009; Irinyi et al., 2009) which causes major economic losses in the vegetable production, especially in the tomato production in glass houses and plastic tunnels (Jovanchev, 2005; Kuzmanovska, 2011). The most important means in controlling of the gray mold was by using chemical treatments (Leroux et al., 2002).

Because of that, the aim of this study was to investigate the possibility of controlling *Botrytis cinerea* in the Republic of Macedonia by biological treatments with the biofungicide Serenade®Max and the possibility of replacing the chemical with biological treatments, in order to achieve healthier tomato production.

Materials and methods

The research was conducted during 2012 on tomato plants in two regions: Valandovo (hybrid Evolution), in four variants (Table 1) and in Strumica (v. Dabile; hybrid Mateas), in three variants (Table 2).

Table 1. Variants represented in Valandovo region

Variant no.	Fungicide and formulation	Active ingredient	Content of a. i.	Producer	Dosage
1	SERENADE MAX	Bacillus subtilis tip QST 713	5,13 x 10 CFU/gr	BASF SE Germany	4 kg/ha
2	SIGNUM (Standard fungicide treatment)	boscalid+ pyraclostrobin	267 +67g/kg	BASF SE Germany	1,5 kg/ha
3	TELDOR (standard fungicide treatment)	Fenhexamid	500 g/l	Bayer CropScience	1,5 l/ha
4	Control	Untreated			

In the Valandovo region each variant consisted of 1450 tomato plants, on an experimental block of 290 m². From the second half of November till the second half of December five fungicide treatments with Serenade®Max were performed (20.11., 27.11., 04.12., 12.12. and 17.12.). The standard fungicide treatments were also performed on the same dates. The evaluation of the fungicide efficacy was conducted on 28.12. The treatments were performed from when 80% of the tomato flowers were in bloom, till the beginning of ripening.

Table 2. Variants represented in Strumica region

Variant no.	Fungicide and formulation	Active ingredient	Content of a. i.	Producer	Dosage
1	SERENADE MAX	Bacillus subtilis tip QST 713	5,13 x 10 CFU/gr	BASF SE Germany	4 kg/ha
2	SIGNUM (Standard fungicide treatment)	boscalid+ pyraclostrobin	267 +67g/kg	BASF SE Germany	1,5 kg/ha
3	Control	Untreated			

In the Strumica region each variant consisted of 50 tomato plants in two repetitions, on an experimental block of 20 m². From the second half of September till the second half of October four fungicide treatments with Serenade®Max were performed (19.09., 26.09., 04.10. and 12.10.). The standard fungicide treatments were also performed on the same dates. In both of the tested regions, the infection intensity was calculated by counting of the total number of healthy and infected plants in the variants and determining percentages of infection. The fungicide efficacy was evaluated by the formula of Abbott (1925).

Results and discussion

The results of the fungicide efficacy obtained during the trials in the Valandovo region are presented in Table 3.

Table 3. Efficacy of the biofungicide Serenade®Max in controlling of *Botrytis cinerea* on tomato in the Valandovo region in 2012

No	Variant	Infection Intensity [%]	Fungicide efficacy [%]
1	SERENADE MAX	0,8	87,7
Standard Fungicide Treatment			
2	SIGNUM	0,4	93,8
3	TELDOR	3,1	52,3
4	Control	6,5	/

From the results obtained in this study, it can be observed that the biofungicide Serenade®Max used in the examined dosage of 4 kg/ha in the area around Valandovo showed high efficacy in controlling of *Botrytis cinerea* – the cause of gray mold disease on tomato. In the variant where control with this biofungicide was performed, 87,7% protection was observed, with infection intensity of 0,8%. It is important to emphasize that in this region in all of the tested variants, only symptoms of stem canker were observed, which the most devastating form of this disease is. This form of *B. cinerea* can cause plant dying (Kuzmanovska, 2011). The observed high fungicide efficacy of 87,7%, when the infection intensity in the control variant was 6,5%, confirms that Serenade®Max is very effective biofungicide in controlling of gray mold disease on tomato. The high efficacy of the biofungicide Serenade®Max was achieved with well-timed and quality application, emphasizing its preventive mode of action. No negative effects were detected during the usage of the biofungicide Serenade®Max on the tomato plants. The results for the biofungicide efficacy of Serenade®Max determined during the trials in the Strumica region are presented in Table 4.

Table 4. Efficacy of the biofungicide Serenade®Max in controlling of *Botrytis cinerea* on tomato in the Strumica region in 2012

No	Variant	Infection Intensity [%]	Fungicide efficacy [%]
1	SERENADE MAX	0,4	87,5
Standard Fungicide Treatment			
2	SIGNUM	0,08	97,5
3	Control	3,2	/

From the results obtained in the studied area around Strumica, it can be observed that the biofungicide Serenade®Max used in the examined dosage of 4 kg/ha showed high efficacy in controlling of *Botrytis cinerea* on tomato. In the Strumica region, highly effective control of the gray mold disease was achieved, with infection intensity of only 0,4%. It is significant to point out that in all of the tested variants in this region, symptoms of gray mold were observed solely on the fruits. The observed high fungicide efficacy of 87,5%, when the infection intensity in the control variant was 3,2%, demonstrates that Serenade®Max is very effective biofungicide in controlling of *Botrytis cinerea* – the cause of gray mold disease on tomato. The high efficacy of the biofungicide Serenade®Max was achieved with well-timed and quality application, emphasizing its preventive mode of action. No negative effects were detected during the usage of the biofungicide Serenade®Max on the tomato plants.

Conclusions

The biofungicide Serenade®Max used in the examined dosage of 4 kg/ha, showed high efficacy in controlling of *Botrytis cinerea* – the cause of gray mold disease on tomato in both of the examined regions. In the Strumica region, highly effective control of the gray mold disease was achieved, with infection intensity of only 0,4%. It is significant to point out that in all of the tested variants in this region, symptoms of gray mold were observed only on the fruits. The observed high fungicide efficacy of 87,5%, when the infection intensity in the control variant was 3,2%, demonstrates that Serenade®Max is very effective biofungicide in controlling of *Botrytis cinerea*. In the Valandovo region, a very effective control of the gray mold disease was also attained, with infection intensity of 0,8%. Unlike the other tested region, in this region in all of the tested variants, only symptoms of stem canker were observed, which is the most devastating form of this disease which causes plant dying. The observed high fungicide efficacy of 87,7%, when the infection intensity in the control variant was 6,5%, coordinates with the results obtained from the trial in the Strumica region which is that Serenade®Max is very effective biofungicide in controlling of gray mold disease on tomato. The high efficacy of the biofungicide Serenade®Max was achieved with well-timed and quality application, emphasizing its preventive mode of action. No negative effects were detected during the usage of the biofungicide Serenade®Max on the tomato plants.

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SECTION 4. FOOD QUALITY AND SAFETY

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THE PRESENCE OF ENTEROBACTERIACEAE IN INDUSTRIAL YOGURT NEAR THE EXPIRY DATE

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Abstract

Degradation of food is a major economic, worldwide problem. Only through microbial activity, about a quarter of the world's food supply is lost. The microbiological quality of milk and milk products are affected by the bacterial content of raw milk, the processing conditions, and the recontamination after the heat treatment. Unwanted microorganisms that can cause spoiling of dairy products include bacteria. Yogurt production usually requires a special attention to the quality of the milk source, the right thermal process, in order to benefit a safe and healthy final product. Bacteriological analyzes are those which determine the contamination of this product, with microorganisms such as *Enterobacteriaceae*, therefore the aim of this study was the isolation and identification of these microorganisms in yogurt samples taken from Tirana market. From January 2014 to December 2014, a total of 280 samples were taken from four different companies and analyzed for the presence of *Enterobacteriaceae*. The samples were taken randomly in Tirana market and their analyses were carried out at Food Safety and Veterinary Institute (FSVI), according to the standard method ISO 21528-2:2004. Overall 280 samples, taken from four different companies, 103 of them resulted positive to *Enterobacteriaceae* but negative to *E.Coli*. Yogurt is a product that is constantly studied for its health benefits, but a poor hygiene during its processing, makes it a contaminated product, harmful to public health.

Key words: *Enterobacteriaceae*, yogurt, hygiene, milk

Introduction

Yogurt is a food produced by bacterial fermentation of milk. The bacteria used to make yogurt are known as "yogurt cultures". Fermentation of lactose by these bacteria produces lactic acid, which acts on milk protein to give yogurt its texture and characteristic tang (Zourari et al., 1992; Adolfsson, 2004; Tamine et al., 2004). The first products of milk have the origin from Asia, Africa, the Middle East and North Europe. Yogurt is nutritionally rich in protein, calcium, riboflavin, vitamin B6, and vitamin B12. It has nutritional benefits beyond those of milk, namely due to its probiotics and it is easily digested. It is one of the most important products consumed in our country and worldwide. To obtain a healthy and nutritional product, it is necessary primarily to monitor the microbiological composition of raw milk. Poor microbiological quality of milk, bacterial contamination during the production, inappropriate storage and lack of hygiene in the

processing environment will affect the quality and microbiological content and his lifespan on the market (Cakiroglu, 1997; Duru et al., 1981; Oz, 1990; Sezgin 1979). Bacterial contamination comes also from the water source, which is a component key of the process. *Enterobacteriaceae* family is a large group, belonging to Gram-negative bacteria, non-spore-forming, facultative anaerobic and vary in length 1-5 μm , which are commonly used as indicators for the evaluation of general hygiene of food products. Members of this family are widely distributed, although some of these genres are less harmful, others are important pathogens for humans as well as animals. Members of this family are responsible for a variety of diseases originating from food products (Yldez, 2009). The purpose of this study was to determine the levels of *Enterobacteriaceae* in the yogurt samples collected from Tirana market. Each sample in this study was randomly collected and then further analyzed according to the standard method ISO 21528-2: 2004.

Materials and methods

A total of 280 samples were taken from four different production companies. The sampling was made randomly at Tirana market, during the period January 2014 – December 2014. Each company was described by letters. It were included 74 samples from the Company A, 80 samples from Company B, 63 samples analyzed from Company C and 63 samples from Company D. All the samples were transported with refrigerator boxes at 4°C to the laboratory at the Food Safety and Veterinary Institute (FSVI), were analyzed for the presence of *Enterobacteriaceae* family. The method used is based on the standard method ISO 21528-2: 2004 for the identification of *Enterobacteriaceae* in yogurt. In aseptic conditions, using sterile pipettes, were taken for analysis 1 ml from each sample and further were inoculated in the selective agar media. 10 ml of VRBG agar, which was pre heated in water bath at temperature 44-47 °C, was poured into each Petri dish. The time interval from the moment of the preparation of the media to the distribution on plates did not exceed 15 min. We conducted careful horizontal movement of the plates in order to mix better the inoculums with the media and let them cool in the room temperature, and then incubated them in 37 °C for 24 $\pm$ 2 hours. After the incubation period, we choose to count the plates with two successive dilutions, containing at least from 15 to 300 typical colonies. If colonies had occupied less than half of the surface plate, then was conducted the count of the clear zone. To calculate the number of microorganisms present in the samples, it was conducted by conducting successive decimal dilutions. Colonies typically appear in red to pink or purple color (with or without an opalescent ring).

Results and discussion

Yeasts are considered a natural yoghurt pollutant. However, many reports indicate bacterial contamination of yogurt. In total it was sampled and analyzed 280 samples, taken randomly from four companies in Tirana market. Out of 74 samples taken from company A, 24 of them resulted positive, with an average load of 3.5×10^3 colony/gr. Compared to those of company B, of which 80 samples of yogurt were analyzed, 40 of them resulted in a greater load with *Enterobacteriaceae*. Out of 63 samples from company C, 18 of them resulted positive, with a lower bacterial load, 3.5×10^3 colony / g, compared with other companies. From company D, with 63 samples, only 21 of them resulted positive. According to other bibliographic data, the results are also seen in other studies. The results

are also seen in other studies. According to Al-Diasty, M.E. and Al Kaseh, M.R. (2007) study, which was conducted in Libya, it resulted that the microbial load was higher, compared to our study. The microbial load in yogurt, in that study ranges with an average of $5 \times 10^4 \pm 1.2 \times 10^4$. This microbial load was present in the milk and then in its product, yogurt.



Figure 1. Sampling in yogurt with average load *Enterobacteriaceae* in Red bile glucose agar (VRBG)



Figure 2. Sampling at the peak of the *Enterobacteriaceae* colony in (VRBG)

The presence of *Enterobacteriaceae* was observed in the first strands of foods, it came as a result of direct fecal contamination of materials (Desmaures et al., 1997; Stewart et al., 2002). Enterobacter in yogurt can come as a result of wrong hygiene during the packaging process, contamination of raw materials and other ingredients and the efficiency during processing. High microbial loads present in Petri dishes, are indicators of the sanitary quality and food safety. Based on different literature, our analysis resulted positive due to contamination that comes from poor hygienic manipulation during the process.

Conclusions

- This study indicated that the family members of *Entebacteriaceae* were present in industrial yogurt of Tirana market.
- The results obtained in this study demonstrate that the positive cases of enterobacteria, resulted in higher load on company B, 4.2×10^3 colony / g, compared with the company A.
- Microbial contamination leads not only in the decay of the product, but also show that the presence of these pathogens pose a risk to public health
- Our results clearly show that the quality of yogurt sampled from different companies resulted in high microbial load.

- The presence of *Enterobacteriaceae* colonies comes as a result of raw product contamination, a poor hygiene of utensils, equipment and packaging thereof.

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IMPROVING THE OXIDATIVE STABILITY OF FATS IN BURGERS

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Abstract

The effects of using a sage (*Salvia officinalis*) both in its dry form and as an extract have been examined, as well as the spice mixture of oregano (*Origanum viridis*) and winter savory (*Satureja Montana*) on the oxidative stability of fats, sensory characteristics, pH-values, weight loss during grilling and microbiological profile of burgers. Burgers, produced according to the common technological procedure, have been used in examinations. Five groups of products have been made: R- referent products without additives; R/B+A products with additives, R/S products with *Salvia officinalis* added in its dried form (0.2 %); R/Se products with 0.05% of *Salvia officinalis* extract added; R/O products with commercial spice mixture - oregano plus added (0.2%). Following examinations have been done: pH-value measurement (at 3 and 7 days after production), grilling weight loss (at 3 and 7 days after production), sensory characteristics (at 3 and 7 days after production), microbiological analysis of burgers (at 1 and 7 days after production) and determination the level of fats oxidation – TBARS test at 7 days after production (the products have been kept at a temperature of 0-4° C), and 90 days after production (the products have been kept at a temperature of -18° C). The lowest pH-value (5.83), at 7 days after production has been determined in group R\Se, while the highest pH-value (5.97) has been determined in group R\B+A, which indicates shorter shelf life of the product. The R/Se products have the highest (14,25%) grilling weight loss. The lowest pH-value, 7 days after production, has been measured in this group which coincides with the statement that as the pH-value decreases, the grilling weight loss increases. At 7 days after production, the R/Se products are claimed to be more sensory acceptable than the rest of the groups of products and they have been the highest evaluated by taste panelists. The R/O group of products has been given the best results in microbiological analysis, which confirms the antimicrobial activity of the spice products. This group has the lowest level of fats oxidation, or the lowest content of malonaldehyde (only 0.567 mg/kg).

Key words: Oxidative stability of fats, sage, oregano, burgers.

Introduction

Fats are important food component. They influence the nutritive and energetic value of the food and its sensory characteristics. It's a fact that the lipids carry the aroma components

of the food, so it is considered that foods with higher levels of lipids taste better and have more intense aroma, which makes them more attractive for the consumers.

Fats and polyunsaturated fatty acids are generally susceptible to changes as hydrolysis and oxidation. Oxidation in fats is an autocatalytic process that happens with food and in biological membranes, which leads to significant impairment of quality of food (Eriksson and Na, 1995). The process of lipid oxidation is influenced by several factors, among which the most important is the presence of polyunsaturated fatty acids, which may be an initiating factor in the oxidative process. The level of lipid oxidation can be increased by the damage of muscle membranes during deboning, meat grinding and cooking (Ladikos and Lougovois, 1990). Lipid oxidation is the leading cause of deterioration of muscle foods. The products of the process of degradation of fats must be considered because of their negative effect on human health. Modern food technology pays great attention to the protection of fats from oxidation and improvement of their oxidative stability. There are many ways to achieve positive results, such as reducing the ambient temperature, reducing the contact surface with air, elimination of factors that support the oxidation and the addition of antioxidants, which is the most effective way of preventing lipid oxidation (Bystrický and Dičáková, 1998). Antioxidants are used to prolong the shelf life of food products, and to ensure their health security. They interfere in the oxidation of fat and other oxidative labile substances as follows:

- react with free radicals (primary antioxidants) or decrease created hydroperoxide (secondary antioxidants)
- they are encapsulated in compounds with metals that catalyze oxidation
- eliminate oxygen

For this purpose the meat industry, and in general in food industry, there is a mass use of additives of chemical origin. The awareness of today's consumer is on a higher level, because the new customer demands less processed foods with a small amount of additives or without use of additives, this can only be achieved by introducing new innovations in the meat industry (Holzapfel et al., 1995). It includes replacement of chemical additives by bio conservatives and spices, which corresponds to modern trends of healthy food.

The purpose of this paper is to determine the effects of using sage (*Salvia officinalis*) both in its dry form and as an extract, as well as the spice mixture of oregano (*Origanum viridis*) and winter savory (*Satureja Montana*) on the oxidative stability of fats, sensory characteristics, pH-values, weight loss during grilling and microbiological profile of burgers.

Materials and methods

Materials

Burgers are used for the experiment, which are produced in the meat industry "Promes" in Skopje. They are produced by common technological procedure applied to the plant for processing meat.

The composition of raw burgers is given in Table 1. After proper treatment, a homogenous mixture of burgers is obtained. Five product groups have been made, as follows:

R - reference products, no additives added;

R / B + A - products with additives [*Bombal* (E262-sodium acetate, E331-sodium citrate, E315- isoascorbic acid, E316-sodium erythroate)] and antioxidants [E300-ascorbic acid, E316-sodium erythroate, E330- citric acid];

R / S - products with sage (*Salvia officinalis*) in a dry form;

R / Se - products with sage extract (*Salvia officinalis*);

R / O - products with spice preparation of *Oregano plus*TM without bombal and reducing agents.

Table 1. Composition of raw burgers

Raw material	Quantity of raw materials (%)
Beef	65 (70% fresh and 30% frozen)
Chicken gut	12
Textured soy	10
Onion	8
Soy flour	4
Soy isolate	1
Additives and spices	
Salt	1,7
Polyphosphates	0,3
Spices	0,45

In this experiment, sage (*Salvia officinalis*) was used in dry and ground form, added in a concentration of 0.2% and an extract of sage (*Salvia officinalis*), added in a concentration of 0.05% in the mixture for the burgers. Also a spice preparation *Oregano plus*TM, from Alkaloid Skopje, was used. The product is a spicy blend of oregano (*Origanum viridis*) and savory (*Satureja montana*) in dry and ground form. The homogenous mixture is added to the burgers in a concentration of 0.2%.

Methods

In this study the following methods are used:

Measurement of pH

The measurement of pH is performed on the mixture for burgers 3 or 7 days after production the burgers. The pH was measured by digital pH-meter. The pH- meter brand is pH-540 GLP.

Weight loss during grilling

Calculating weight loss during grilling is performed 3 and 7 days after manufacture. Weight loss during grilling the burgers is calculated by subtracting the weight of the finished product (heat treated) from its raw mass. The resulting mass is expressed in percent relative to the weight of thermally untreated product.

Examination the sensory characteristics

Examination the sensory characteristics (appearance, color, smell, taste, texture and consistency) is performed at 3 or 7 days after manufacturing. The evaluation of sensory properties of the finished products is performed according to the method of scoring (Radovanović and Popov-Rajlić, 2000/2001), by a taste panel of 5 members. The Sensory evaluation includes five attributes: appearance, cross-section appearance, consistency, smell and taste. For each of these properties there is a coefficient of importance: appearance - 1, cross-section appearance - 4 consistency - 3 Color - 3 fragrance - 4, and taste - 5. The evaluated properties are evaluated on a scale of 1 to 5 (1- bad, 2-poor; 3-

good, 4-very good 5-excellent). The assessment for each property is multiplied by the coefficient of importance, and the resulting value is entered in the table. The entered values are collected and divided by the sum of the coefficients (20). The resulting value represents the weighted average, that weighted score - a general assessment of the quality of the tested burger. Moreover, the percentage is calculated.

Microbiological analysis of the burgers

Samples are taken at 1 day and 7 days after manufacture. During the experiment for the microbiological analysis the following standard analytical methods were used: *Campylobacter* (ISO 10272-2), *Yersinia enterocolitica* (ISO 10273), *Salmonella* spp. (ISO 6579), *Listeria monocytogenes* (ISO 11290-1), *E.coli* O157:H7 (ISO 16654), *Enterobacteriaceae* (ISO 21528-2) and ISO 4833.

Determination the level of fats oxidation – TBARS test

Determining the extent of oxidation of the lipids in the burger using the TBARS (Thiobarbituric Acid Reactive Substances) test is performed 7 days after production (products are stored at a temperature of 0 - 4°C) and 90 days after production (products are stored at a temperature of - 18°C). Before the TBARS number is determined burgers are grilled, and after cooling at a room temperature, they have been stored overnight in a refrigerator at a temperature of 0-4°C.

The method of thiobarbituric acid is one of the most reliable tests used to determine the degree of oxidation of the lipids. This method is based on the reaction of aldehydes, which are formed as side products in the oxidation of lipids, with thiobarbituric acid, where as product a complex compound is obtained and it's colored pink. By measuring the absorbance of the complex compound the degree of oxidation of the lipids can be directly determined.

TBARS - test is performed according to the method of Tarladgis et al. (1960), modified by Shahidi et al. (1987), as follows:

In a distillation balloon of 500 ml 10 g of homogenized sample were measured, 97,5 ml of distilled water were added, 2,5 ml 4N HCl, some glass beads and small rod coated with Teflon. The balloon is placed on a magnetic stirrer and the content is mixed for 2 minutes at 1200 rpm. Then the bubble content is distilled with boiling water vapor and distillate is collected in a 50 ml glass flask. Distillation takes about 10 minutes. In glass flask 5 ml are separated from 25 ml of the distillate and 5 ml 0,02 M aqueous solution of thiobarbituric acid (TBARS) are added. The glass flask are closed with a stopper and heated in boiling water bath for a period of 35 minutes. After cooling the absorbance is measured, at a wavelength of 532 nm, using a spectrophotometer JENWAY 6305th

To convert the absorbance units into TBARS-numbers, defined as the concentration of malonaldehyde in mg / kg, received absorbance units are multiplied by a factor of 8,1 (Shahidi et al. 1983).

Results and discussion

Measurement of pH value

The results of the measurement of pH at 3 and 7 days after manufacturing are shown in Table 2.

Table 2. pH value of the products

SECTION 4. FOOD QUALITY AND SAFETY

The time elapsed since the date of production	Product groups				
	R	R\B+A	R\S	R\Se	R\O
3 days	6.125	6.145	6.14	6.1425	6.13
7 days	5.966	5.972	5.884	5.83	5.862

After the measurement of the pH of the product at 3 days elapsed, the lowest output value (6.125) has the reference group-R which has no additives. But the difference in pH- value between the groups is negligible small.

The lowest pH value (5.83) at day 7. After production has the group product R \ Se. The highest pH value (5.972) has got the group of products R\B+A.

The lower pH- value is a factor that affects the shelf life stability of the product.

Weight loss during grilling

The results of the weight loss during grilling are shown in Table 3., expressed in percents (%).

Table 3. Weight loss during grilling at 3 and 7 days after production.

The time elapsed since the date of production	Weight loss during grilling				
	R	R\B+A	R\S	R\Se	R\O
3 days	11.283	9.92	8.7875	9.6475	13.6975
7 days	14.225	12.2375	13.24	14.2525	11.2275

From the weight loss measurement during grilling at 3 days after production (Table 3) it can be concluded that the lowest loss in mass has got the R/S group (8.7875%). The highest weight loss during grilling (13.6975%) was observed in the group R\O. 7 days after production weight loss during grilling is highest in the R\Se group (14.2525%).

Sensory analysis

The results of the sensory analysis 3 days after manufacture are shown in table 4.

Table 4. Sensory evaluation of the products 3 days after production.

Selected sensory characteristics	KB	Product groups									
		R		R\B+A		R\S		R\Se		R\O	
		O	K	O	K	O	K	O	K	O	K
External appearance	1	3	3	3.9	3.9	3.7	3.7	3.9	3.9	3.7	3.7
Cross-section appearance	4	3.6	14.4	4.2	16.8	3.4	13.6	3.6	14.4	3.6	14.4
Texture	3	3.3	9.9	4	12	3.7	11.1	3.2	9.6	3.7	11.1
Color	3	3	9	4	12	3	9	3.3	9.9	3.4	10.2
Smell	4	3.5	14	3.9	15.6	2.5	10	2.9	11.6	3.7	14.8
Taste	5	3.5	17.5	4.1	20.5	2.8	14	2.7	13.5	3.9	19.5
Total KB	20										
Average value		3.3		4.1		3.2		3.2		3.6	
% from the maximal possible quality			67.8		80.8		61.4		62.9		73.7

(KB = coefficient of importance; O = grade; K = correction value)

After the examinations of sensory properties of five specific product groups at three days after their production, evaluated sensory properties that meet the most (80.8% of the

maximum possible quality) are those of burgers with additives. In contrast worst (61.4% of the maximum possible quality) was evaluated in product group (R \ S).

The results of the sensory analysis 7 days after manufacturing are shown in Table 5.

Table 5. Sensory evaluation of the products 7 days after production.

Selected sensory characteristics	KB	Product groups									
		R		R\B+A		R\S		R\Se		R\O	
		O	K	O	K	O	K	O	K	O	K
External appearance	1	2.75	2.8	3.9	3.9	4.25	4.25	3.9	3.9	3.9	3.9
Cross-section appearance	4	2.5	10	3.75	15	3.9	15.5	4.1	16.5	3.9	15.5
Texture	3	2.25	6.8	4	12	4	12	4.2	12.8	4.25	12.8
Color	3	2.6	7.9	3.7	10.9	4.4	13.1	4	12	3.9	11.6
Smell	4	2.4	9.5	3.7	14.5	4	16	4	16	4.25	17
Taste	5	2.25	11.3	3.75	18.75	4.1	20.6	4.1	20.6	3.75	18.8
Total KB	20										
Average value		2.46		3.8		4.1		4.1		3.9	
% from the maximal possible quality			48.1		75		81.5		81.75		79.6

(KB = coefficient of importance; O = grade; K = correction value)

According to the results shown in Table 5, 7 days after production sensory properties that meet the most (81.75% of the maximum possible quality) are those in which extract of sage was added (R \ Se), while the lowest (48.1% of the maximum possible quality) was evaluated in a reference group of products (R).

Microbiological analysis

The results of the microbiological analysis after 1. and 7. day after production are shown in Tables 6 and 7.

Microbiological testing of the reference product group and the control groups (R; R\B+A; R\S; R\Se; R\O) are conducted at the Veterinary Institute in Skopje on .1 and 7. Day after production.

SECTION 4. FOOD QUALITY AND SAFETY

Table 6. Number of bacteria after the 1st day of production

Bacteria	Groups of burgers				
	R	R\B+A	R\S	R\Se	R\O
	1 st day	1 st day	1 st day	1 st day	1 st day
<i>Campylobacter</i>	-	-	-	-	-
<i>Yersinia enterocolitica</i>	-	-	-	-	-
<i>Salmonella spp.</i>	-	-	-	-	-
<i>Listeria monocytogenes</i>	-	-	-	-	-
<i>E.coli</i> O157:H7	-	-	-	-	-
<i>Enterobacteriaceae</i>	4x10 ⁶	4x10 ⁴	32x10 ³	40x10 ³	2x10 ⁴
Total number of aerobic mesophilic bacteria (cfu/g)	11x10 ⁵	11x10 ⁴	32x10 ⁴	38x10 ⁴	2x10 ⁴

Table 7. Number of bacteria after the 7th day of production

Bacteria	Groups of burgers				
	R	R\B+A	R\S	R\Se	R\O
	7 th day	7 th day	7 th day	7 th day	7 th day
<i>Campylobacter</i>	-	-	-	-	-
<i>Yersinia enterocolitica</i>	-	-	-	-	-
<i>Salmonella spp.</i>	-	-	-	-	-
<i>Listeria monocytogenes</i>	-	-	-	-	-
<i>E.coli</i> O157:H7	-	-	-	-	-
<i>Enterobacteriaceae</i>	45x10 ⁷	45x10 ⁵	30x10 ³	41x10 ³	6x10 ³
Total number of aerobic mesophilic bacteria (cfu/g)	15x10 ⁷	2x10 ⁵	23x10 ³	40x10 ⁴	2x10 ⁴

From the results presented it can be seen that the reference group (R) product provides the worst results in microbiological analysis in both time interval, 1 and 7 days after production. In contrast to this group - R\O, gives the best results. It can be seen from the results that the presence of particular spicy mixture reduces the presence of Enterobacteriaceae (facultative pathological anaerobic bacteria) from 2x10⁴ cfu/g after the first day of production to 6x10³ cfu/g after the seventh day of the production, which confirms the antimicrobial effect of spicy products.

Determination of the level of oxidation of fats (TBARS- test)

The results of the determination of the level of oxidation of fats (TBARS- test) are shown in Table 8 and the oscillations of the values are presented in the Diagram 1.

Table 8. Content of malondialdehyde (mg / kg)

Groups	TBARS - value	
	(7 days)	(90 days)
R	0.5751	0.7047
R/B+A	0.324	0.9801
R/S	0.3807	0.7402
R/Se	0.2916	0.7533
R/O	0.243	0.567

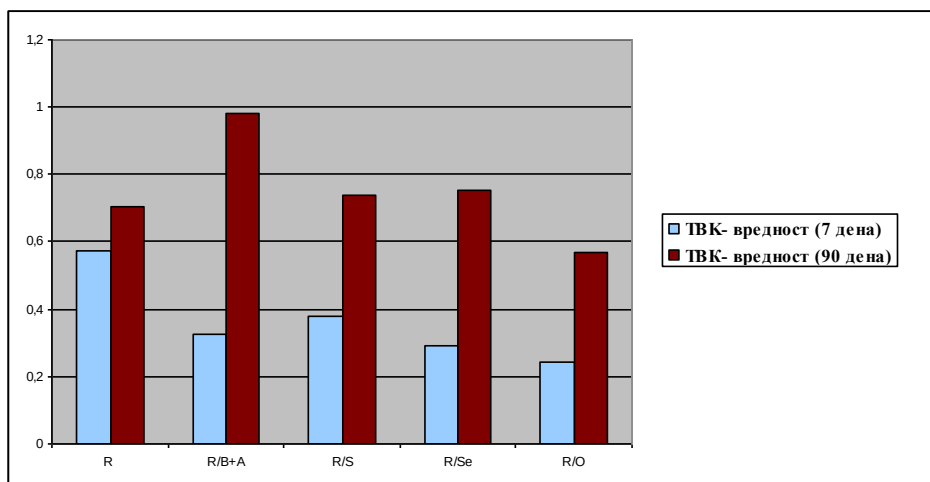


Figure 1. Content of malondialdehyde (mg/kg) (oscillations of the values)

It can be seen from the results of the TBARS- test that 7 days after production the highest values for the content of malonaldehyde have been measured in the reference group products (0,5751 mg / kg), while the lowest value (0,243 mg / kg) was measured in the group R\O.

When performing a measurement of the amount of malonaldehyde using the same method 90 days after production, the results indicated that the highest value (0,9801mg/kg) was measured in the product group R/B+A. The lowest measured value (0,567 mg/kg) remains in the group of products R\O.

The results of determining the degree of oxidation of fats with TBARS- test, show that the lowest value of content of malonaldehyde was measured for the group of products R\O which coincide with literature data, specifically Fasseas et al. (2008) that examined the anti-oxidative effect on Sage and oregano in ground beef.

Conclusion

Based on the results of examination the following conclusions may be adopted:

1. Although the minimum difference in the measured pH values in the tested groups of products was found, however, seven days after manufacturing, the lowest pH value (5.83) was measured in burgers, which contained the extract of sage (R\Se). The highest pH value (5.972) has got the group of products R\B+A, indicating a lower durability of the product. Therefore, the addition of an extract of sage in burgers could be considered as a method to extend the shelf life.
2. Weight loss during grilling is highest among the products with added extract of sage. In this group of products it is also determined the lowest pH value 7 days after production which coincides with the claim that the decrease in pH increases the weight loss during grilling.
3. After the period of 7 days of production, the products in which an extract of sage has been added (R\Se), are sensory more acceptable than other groups and are the highest rated.

4. The R\O product group, burgers with Oregano plus (a mixture of oregano and savory) give the best results in microbiological analysis, which confirmed the antimicrobial effect of spice preparations.
5. The lowest level of fat oxidation, or the lowest content of malonaldehyde (0,567 mg/kg), has the group of products R\O, burgers with spice preparation Oregano plus (mixture of oregano and savory).

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ISOLATION OF *MYCOBACTERIUM BOVIS* FROM SUSPECTED TUBERCULAR LESIONS IN CATTLE

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Abstract

Bovine tuberculosis still remains an endemic infectious disease in cattle and a real threat for human health. Confirmation of diagnosis is made by microscopic examination and culturing in proper microbiologic media. We demonstrated the presence of *Mycobacterium bovis* in microscopic smears stained by Ziehl-Neelsen (ZN) method, and the pathogen was recovered both in solid Lowenstein-Jensen media (LJ) and liquid media (BACTEC system). In addition, there were identified specific changes in pulmonary and lymph nodes lesions. The unification of veterinary services, identification of animals, movement control, institutional cooperation with public health department and other actors is an important challenge for a successful control of the infective disease in general and specifically for bovine tuberculosis.

Key words: bovine tuberculosis, surveillance, slaughter, BACTEC system

Introduction

Bovine tuberculosis is a bacterial contagious disease due by *Mycobacterium bovis* (*M. bovis*) that affect animals and humans. The common and most effective transmission route of *M.bovis* is by aerosol, consequently, typical lesions in cattle are manifested mainly in pulmonary tissue and at regional lymph nodes. Controversially, in affected calves, the lesions primarily are founded at mesenteric lymph nodes as they acquired the infection by suckling infected milk.

Primary source of infection are infected cattle and several wild animals, some of them serve as reservoirs. *M.bovis* is excreted by respiratory excretions, feces, milk, urine and semen (Markey *et al*, 2013). Pathogenesis of disease depends by host, pathogen and environment factors (Quinn *et al.*, 2011).

As infection is established, *M.bovis* multiplies at entry point and reach the regional lymph nodes. In later stage it travel to the thoracic ducts that make possible generalization of the infection (Aiello, 1998). Wax and proteins stimulates a type IV of hypersensitive reaction. Some weeks later T cells produce cytokines and macrophages accumulate at foci of infection. Due to the continuous accumulation of macrophages are formed the tubercles which are surrounded by fibrotic tissue (Gyles *et al.*, 2010; Heymann *et al.*, 2004). In center of tubercular lesions occur caseous necrosis, which in later stage may undergo to calcification. In infected animals within 4 to 5 weeks, delayed hypersensitivity occur, this

is used as a toll for diagnosing of disease either in field conditions (skin test) or laboratory test (INF- γ test). Traditionally in Albania, control of bovine tuberculosis is based on applying the single cervical intra-dermal skin test as a screening purpose followed by simultaneous skin test two months later for confirmation of positive cases during single skin test. Unfortunately, the program cover only a small percentage of cattle population and it is not evidence based on risk. This study is part of a large study and its aim is to evaluate the efficacy of meat inspection in surveillance of bovine tuberculosis. From suspected bovine tuberculosis case during routine meat inspection we demonstrated presence of *M. bovis* by isolation. Typical alternation was examined in microscopic slides prepared on tissues affected.

Materials and methods

Animals: In this study were included two animals: 1) a cow (without ID, unknown origin) slaughtered for meat consumption at a regional slaughterhouse; 2) a calf with unresponsive diarrhea syndrome. Fortunately, the origin of the calf was known and the owner was very interested to confirm the diagnoses and to apply all measures in order to control the disease. After the first doubts, affected organs were submitted to the laboratory of infectious disease at Agricultural University, Faculty of Veterinary Medicine, Tirana. All tissue specimens were carefully examined both macroscopically and microscopically. Approximately 5-10g samples were taken from cow lung tissue and the same amount from calf mesenteric lymph nodes. . We cultured in proper media and prepared microscopic smears. Five-micrometer tissue sections were stained with Hematoxylin - Eosin and examined by light microscopy thankful assistance of experts from histopathology laboratory at Food Safety Institute and Veterinary Institute, Tirana (ISUV)

Isolation of *M. bovis* was done at national reference laboratory, at Human Pulmonary University "Shefqet Ndroqi", Tirana. Specimens for bacteriologic culturing were placed in sterile plastic containers and frozen at -80°C. We used two different medias for isolation Lowenstein-Jensen solid media and in liquid media, by using BACTEC MGIT system (Schiller et al., 2010). All steps recommended for *M.bovis* isolation were followed: sample preparation, decontamination, concentration and inoculation in respective media. After concentration step, microscopic slides were prepared and stained according to ZN method (Quinn et al., 2011).

Results and discussion

Animals were classified as infected if results of either bacteriologic or histological examination were positive. Both suspected cases were classified as infected based on histologic examination and results of bacteriologic determination. During meat inspection lesions of tuberculosis were observed in the lung tissues, tracheobronchial and mediastinal lymph nodes of the cow (Figure 1). As where as in the calf gross lesions were present in mesenterial lymph nodes, spleen, diaphragms, liver and its lymph nodes. (Figure 2).

Typical tubercular lesions were present in both cases, however in cow the lesions seems to be older, larger, well encapsulated, contained a thick greenish yellow exudate and less in number: in calf lesions were smaller, spread in many organs in abdominal cavity and seems to be developed fast and recently. Table 1 are shown affected tissues and organs of cow and calf respectively.

Table 1. Presence of tubercular lesions in two infected cattle with *M.bovis*

	Tissue with tubercular lesions	Cow	Calf
1	Lungs	+++	Negative
2	Mediastinal lymph nodes	+++	Negative
3	Mammary lymph nodes	Negative	Negative
4	Mesenteric lymph nodes	Negative	+++
5	Spleen	Negative	+++
6	Liver port	Negative	+++
7	Diaphragm	Negative	+++

Presence of tubercular lesions in calf and its localization indicate that most likely source of infection is colostrums and/or milk. It is assumed that mother could be infected by *M.bovis*. The milk from infected cow represents a serious concern for the public health.

Histological examination

Histo-pathological examination was performed on smears prepared from affected tissue. (Palmer et al., 2006).

The positive results were considered if microscopic changes were characteristic for tuberculosis, such as presence of cellular infiltration, granulomas, with or without caseous necrosis and or mineralization etc (Figure 3). Presence of granulomatous lesions was observed in the lymph nodes, and acid fast bacteria were present in some small lesions. There was no evidence of caseous necrosis or mineralization.

In summary, microscopically most typical finding were: different size of granulomas surrounded by fibrotic capsule, large number of epithelial cells, giant cells and cellular infiltration (Figure 4).



Figure1. Tubercular lesions in the mediastinal lymph nodes of cow

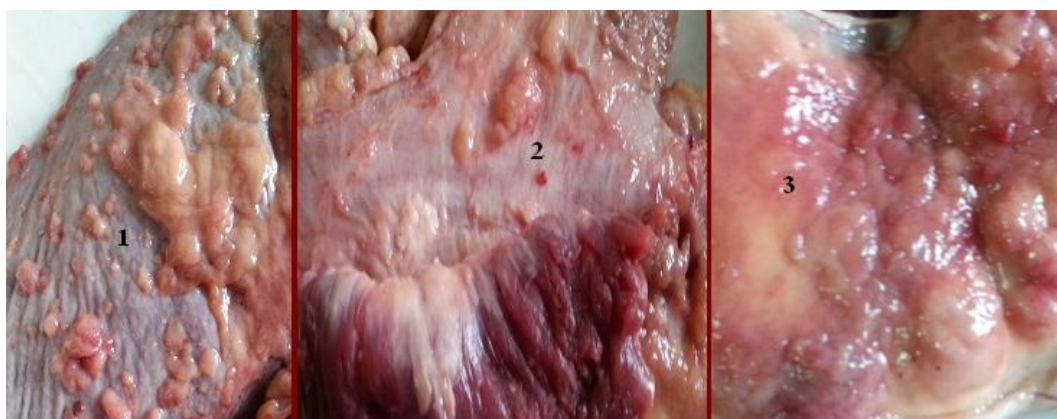


Figure 2. A generalized, miliary form of bovine tuberculosis in calf. Lesions in different organs of the abdominal cavity 1. Spleen 2. Crura diafragmatica 3. Abdominal surface of diaphragm

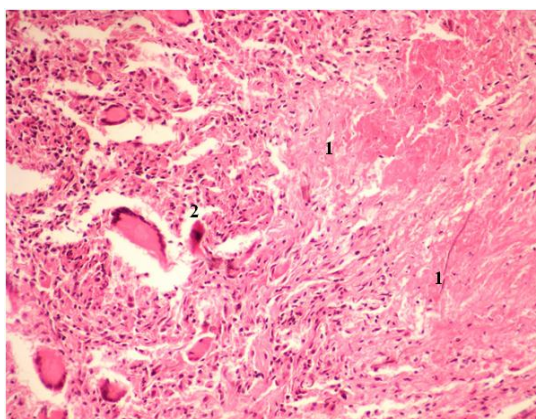


Figure 3. Histological section from pulmonary tissue 1. Tubercular granuloma with necrotic zones 2. Langhans cell type, presence of Giant cells

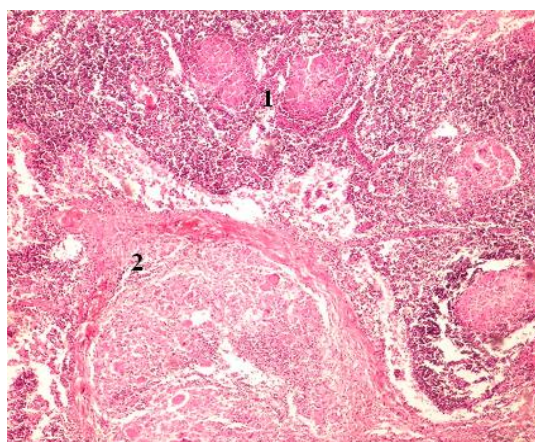


Figure 4. Section from mesenteric lymph node 1. Fibrotic granuloma 2. Capsulated granuloma with caseous necrosis

Mycobacterium bovis isolation

M. bovis was recovered from the tracheobronchial lymph nodes of cow and from the mesenteric lymph nodes of the calf (Figure 5). *M. bovis* was isolated in both medias, respectively after eight weeks in LJ media and five weeks in BACTEC system (Figure 6 and 7).

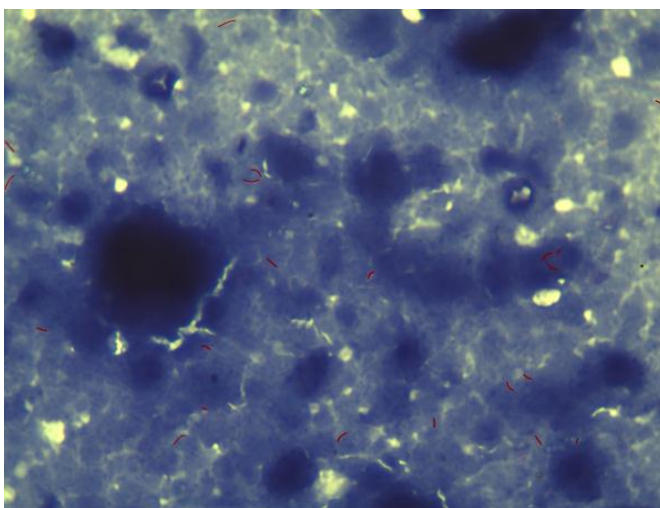


Figure 5. Microscopic view of *M.bovis* stained with Ziehl-Neelsen



Figure 6. *M.bovis* grown in Lowenstein- Jensen media



Figure 7. Growth of *Mycobacterium bovis* in liquid media, BACTEC System BGIT 960

Conclusions

In conclusions, we confirmed the presence of *M. bovis* on suspected organs of cow and calf. The pathogen was isolated in Lowenstein-Jensen media and liquid media (BACTEC system). In BACTEC system *M. bovis* was grown faster. Rigorous meat inspection in slaughterhouse is crucial for detecting suspected cases of bovine tuberculosis. They must be followed by further confirmatory laboratory tests. Collaboration with human health is necessary for control of disease and sharing expertise under one health philosophy. In addition, all suspected cases must be followed up and active surveillance have to be applied in epidemiologic units.

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COMPARISON OF THE NUTRITIONAL COMPOSITION OF FRESH AND DRIED FRUITS FROM SEVERAL APPLE VARIETIES

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Abstract

Apple as fruit is especially important to be consumed in the diet, as fresh well as dried because of the abundance of nutrients. Apple fruits belong to the group of productions with low calories value, and are rich in many sugars, vitamins, organic acids and mineral matters. The fruit is the best for fresh consuming, but on the other hand, because of its nature and season character, it is accessed to preserving with drying. In this way, the apples can be used for a longer period. The aim of our exploration was comparing the differences of nutrients between fresh and dried apple fruits from the cultivars: ajdared, fudzi and zlaten delishes. By the chemical content, were following the changes of total dry matters, total sugars, organic acids, vitamin C and mineral matters. According to analysis, it is determined that the content of total dry matters in the fresh fruits is ranged from 13.10 % in zlaten delishes cultivar to 14.15 % in fudzi cultivar. In the dry fruits, the highest content of total dry matters is determined in the ajdared cultivar (94,50 %). The highest content of total sugars in both of fresh and dried fruits is determined in the fudzi cultivar, 26.50 % in fresh fruits and 57.37 % in dried fruits.

Key words: apple, drying, dry matters, sugars, vitamin C.

Introduction

Drying is one of the oldest methods of food conserving that is widely used in the past (drying on the sun). As a method of conserving was known in the period of ancient Egyptians and others old civilizations (Karakasova et al., 2013). During the drying, water is reducing from the food in amount that allows neither contamination nor taste loss of product. Food must not lose all of the water while that may have negative influence on the ductility and radiation ability (Vereš, 2004).

Different ways of drying provide a wide range of choice among manufacturers regarding the application of the method of drying including the economic aspect is of particular importance for each manufacturer.

A particular advantage of canning drying is that by removing the water from the product, its mass and volume are reduced, and thus the cost of the necessary packaging, transport and storage (Karakasova et al., 2007) that makes this way of preserving much more economical (Stojanova and Karakasov, 2014.).

Basic elements of which depend the speed of drying and the quality of the drying process are the physical characteristics of the heated medium or atmosphere in which the drying and the physical and chemical properties of the product, the thickness of the layer and the drying mode is associated with the characteristics of the dryer. (Никетић-Алексић, 1994).

The purpose of drying is getting a product with a porous structure, good power for rehydration and fewer changes in organoleptic properties depending on the raw material properties (Obradović, 2011).

Dried foods are characterized by a high concentration of dry matter which creates unfavorable conditions for the development of micro-organisms present and the metabolic activity of the enzyme. In this way canned food can be stored (under appropriate conditions) a longer period of time without the risk of spoiling. Dried fruit has a series of advantages compared to fruit that is canned otherwise (freezing, acidification) given that it remains preserved most of the vitamins, minerals and other ingredients, and it also has excellent taste. Of great importance is that in dried fruit do not have to be added chemicals and preservatives that puts dried fruit in a healthy food for humans. Apples as a fruit is characterized with high nutritive properties because it has a significant role in human nutrition.

Humans have long used the apple as food, refreshment and as medicine (Mišić, 1988). Nutritional and healing properties of apple have been known since ancient times of human civilization (Paunović et al., 2010). Apple is one of the leading fruits that is grown in temperate areas of the world. Its handsome looks, crisp flesh and a pleasant sweet taste attracts the attention of consumers (Nour et al., 2010).

Apple fruits are characterized by extremely complex and diverse chemical composition. Dominant features of apples are taste of sweetness and acidity caused by sugar (glucose, fructose and sucrose) and apple acid which are the main components of the apple even the water. Apple is also composed of vitamins and minerals. Water in the fruits of apples is represented by 84 to 86 % (Stojanova and Karakasov, 2014.; Karakasova et al., 2007).

According to (Никетић-Алексић, 1994) from the technological aspect, the fruits of apples should fulfill certain properties: it can be adapted to all types of processing, the distinctive quality features during preservation should not be lost, easy to submit preparatory operations, and during the processing to obtain the less waste.

Significant nutritional composition places the apple in the healthy food that can be consumed throughout the day both in fresh and in dried form. The aim of our research was to compare the nutritional composition of fresh and dried fruits of apple varieties *Golden delicious*, *Idared* and *Fuji* using two different pretreatment.

Materials and methods

In our research, as work materials were used apple fruits from three varieties: *Golden delicious*, *Idared* and *Fuji*.

Golden delicious is considered the most widespread varieties of apples grown in the world. It is winter variety that is picked in the beginning of September and can be stored in appropriated conditions till the end of May. The fruits are large (123 – 280g), with yellow-green surface cover that during storage passes in golden yellow. The fleshy part is yellow, crispy, juicy, aromatic and quite tasty (Кремановић, 1996).

Idared variety is selected in the US in 1947 by crossing between Jonatan X Vagener. It is a highly productive plantation variety that is easy to maintain (Vujevič et al., 2011). The fruits are large (165-189 g). The surface cover is bright red with additional shades of purple-red and dark-red fruit that makes it beautiful and decorative. The fleshy part is white and juicy with sweet - sour taste. *Idared* variety is resistant to low temperatures that allows the fruit to be stored a long time in ordinary warehouses, but it is sensitive to ashtrays (Кремановић, 1996; Мишић 1988; Станђевић, 2002)

Fuji variety is created by cross between Red Delicious X Ralls Janet in horticultural research station in Tohoku, Marioka in Japan. It is selected in 1939 as Tohoku 7, and under the name *Fuji* had been introduced in 1962 (Кипријановски, 2004). In the last ten years it begins to spread in Europe as a highly productive plantation variety. It can be picked in late October. It is resistant to the trays, but is sensitive to fuzikladium and blight. The size of the fruit is medium to large. Surface cover is light yellow that illuminates blurry pink-red through yellow-green color. The fleshy part has a beautiful texture, crunchy, juicy with a pronounced sweet taste and creamy yellow color. The fruits of this variety can be stored at least 6 months or longer. There are a number of colored clones of the standard type of this variety (Gvozdanović, 998, Кипријановски, 2004).

Nutritional properties of the varieties *Golden delicious*, *Idared* and *Fuji* were determined through analysis of the chemical composition of the fresh and the dried apple fruits. The results were obtained as the average of two repetitions. From the chemical composition, the following parameters were determined:

- Total dry matter is determined gravimetrically as residue remaining after drying into the drying oven at 105 °C to constant weight;
- Quantity of moisture obtained by calculation when 100 % will be subtracted value (percentage) of the total dry matter;
- Vitamin C was determined by the Tillmans method, which is based on a redox reaction between L-ascorbic acid and organic colored 2,6 dichlorophenol indophenol;
- Total acids were determined by 0.1 M solution of NaOH, and 1 % solution of phenolphthalein as indicator.
- Mineral substances (total ash) were determined by gravimetric method based on the incineration and burning of the prepared sample at 525 °C ± 25 °C and measuring the resulting residue.
- Total sugars were determined according Lane - Eynon (Lane and Eynon) method based on reduction properties of sugars that, when its heated, reduce divalent copper ion (Cu^{2+}) from Fehling's solution to red precipitate of Cu_2O .

After examining the chemical composition of fresh fruits, was made preparation of the raw material for drying. The preparation of the fruit consists of the following technological operations: washing, removing the stem and seeds lodge, peeling and cutting.

Treating the raw material was made in two variants:

- With 0.1% solution of citric acid for a period of 5 minutes
- Immersion of the feed in the 0.5 % solution of $\text{K}_2\text{S}_2\text{O}_5$ for a period of 3 minutes.
- Drying was made in a ventricular dryer of about 6 to 8 hours at a temperature of 60 °C to 70 °C.

Results and discussion

The results of the chemical composition of three apple varieties *Idared*, *Golden delicious* and *Fuji*, are presented in Table 1 and Table 2.

Table 1. Chemical composition of fresh fruit of Idared, Fuji and Golden delicious

Parameters	Idared	Fuji	Golden delicious
Water (%)	86.14	85.85	86.9
Total dry matters (%)	13.86	14.15	13.10
Total sugars (%)	19.77	26.50	16.81
Organic acids (as citric acid) (%)	0.19	0.09	0.18
Vitamin C (mg / 100 g)	2.37	2.37	1.68
Ash (%)	0.16	0.14	0.18

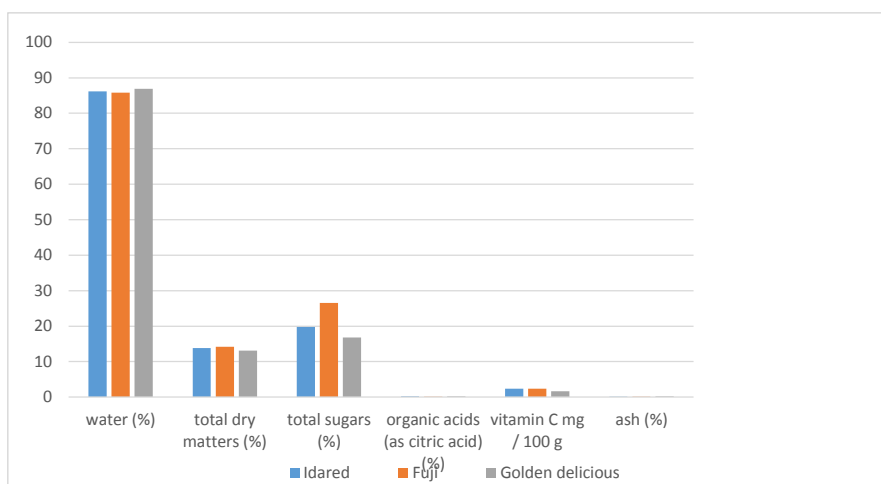


Figure 1. Chemical composition of fresh fruit of Idared, Fuji and Golden delicious

The presented data in Table 1, shows that the fresh fruits from the variety *Golden Delicious* contain higher percent of water compared to the fresh fruits from the varieties *Idared* and *Fuji*. In the variety *Golden delicious* Karakashova et al., (2007) determined 0.49 % ash, 0.16 % total acids and 16.60 % dry matters. According to Stojanova and Karakashov (2014) the variety *Golden delicious* contains 12.21 % dry matters, 0.19 % ash and 22.03 mg/100 g vitamin C. Nour et al., (2010) in the variety *Golden delicious* determined 14.72 % ash, 3.8 mg/100g vitamin C and 11.52 % total sugars. In our research in the fresh fruits from the variety *Golden delicious* was determined 0.18 % ash, 0.18 % organic acids (as citric acid), 13.10 % dry matters, 16.81 % total sugars and 1.68 mg/100g vitamin C. The variety *Idared*, according to Nour et al., (2010) contains 11.4 mg/100g vitamin C and 11.31 % total sugars. The presented data in Table 1 shows that the fresh apple fruits from the variety *Idared* had higher content of organic acids (0.19 %), total dry matters (13.86 %), vitamin C (2.37 mg/100g) and total sugars (19.77 %) compared to the fresh fruits from the variety *Golden delicious*. The lowest content of water (85.85 %), ash (0.14 %) and organic acids (0.09 %) was determined in the fresh fruits from the variety *Fuji*. In this variety was determined the highest content of total acids (26.50 %) compared to the fruits from the varieties *Idared* and *Golden delicious*.

Fresh apple fruits from the varieties *Idared*, *Fuji* and *Golden delicious* after washing, peeling, removing the seeds and seed stock and cutting the pieces, were treated as follows:

- Variant I – fruits treated with 0.1 % solution of citric acid for 5 min
- Variant II – fruits treated with 0.5 % solution of $K_2S_2O_5$ for 3 min

Prepared apple slices were placed on ventricular drier. Drying is performed at a temperature of 60 °C to 70 °C for 6 to 8 hours. After drying was made analysis of the chemical composition of dried apples, and the results are presented in Table 2.

Table 2. Chemical composition of dried fruits from the varieties *Idared*, *Fuji* and *Golden delicious* (variant I).

Parameters	Idared I	Fuji I	Golden delicious I
Water (%)	8.55	7.69	6.74
Total dry matters (%)	91.45	92.31	93.26
Total sugars (%)	54.62	57.37	56.46
Organic acids (as citric acid) (%)	1.93	1.48	1.48
Vitamin C mg / 100 g	6.25	4.82	7.40
Ash (%)	0.82	0.34	1.25

Table 3. Chemical composition of dried fruits from the varieties *Idared*, *Fuji* and *Golden delicious* (variant II).

Parameters	Idared II	Fuji II	Golden delicious II
Water (%)	5.51	5.58	6.29
Total dry matters (%)	94.50	94.42	93.71
Total sugars (%)	51.59	56.79	55.25
Organic acids (as citric acid) (%)	1.81	1.32	1.32
Vitamin C mg / 100 g	21.12	17.56	13.38
Ash (%)	1.35	1.37	1.45

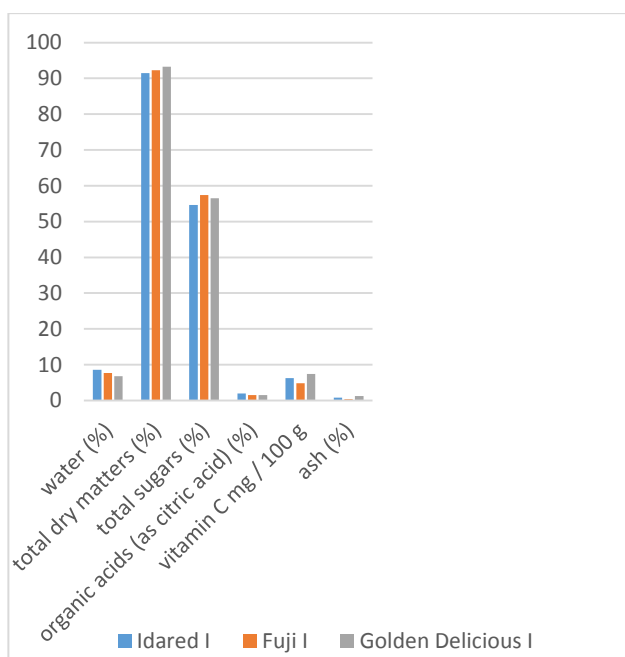


Figure 2. Chemical composition of dried fruits from the varieties *Idared*, *Fuji* and *Golden delicious*. (variant I).

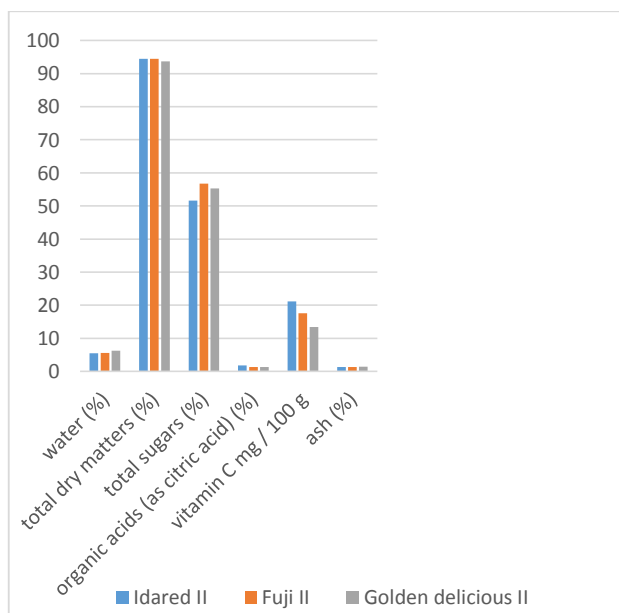


Figure 3. Chemical composition of dried fruits from the varieties *Idared*, *Fuji* and *Golden delicious*. (variant II).

The obtained result from the examination presented in Table 2 and Table 3 shows that dried fruits from the variety *Idared* had the lowest content of water (5.51 %) in the

variant II compared to dried fruits from the variety *Golden delicious*, where the water content was 6.29 % in the variant II to 6.74 % in the variant I. In the variety *Fuji* the content of water was 5.58 % in the variant II to 7.69 % in the variant I. The content of total sugars is the highest in the variety *Fuji* and its value was 57.37 % in the variant I and 56.79 % in the variant II. The highest content of total dry matters was determined in the dried fruit from the variety *Idared* (94.50 %) in variant II. The lower content of ash was determined in dried fruits from the variety *Fuji* (0.34 %) in the variant I. The obtained results from the analyses shows that there were statistical data for significant differences in the content of vitamin C in dried fruits between variant I and variant II. The content in the variant II is higher compared to the variant I. No deviations between variants I and II for the content of organic acids in dried fruits for variety *Fuji* and *Golden delicious* were found (Table 2.) In dried fruits from variety *Idared* was determined difference between variant I (1.39 %) and variant II (1.81 %).

Conclusion

From the obtained results for the comparison of the nutritional composition of fresh and dried apple fruits from the varieties *Idared*, *Fuji* and *Golden delicious*, the following conclusions can be made:

- Fresh fruits from the variety *Golden delicious* had the highest content of water and ash, but the lowest content of total dry matters.
- In the fresh fruits from the variety *Idared* the highest values were determined for the content of vitamin C (2.37 mg/100g) and organic acids (0.19 %).
- The highest content of total sugars and total dry matters was determined in the fresh fruits from the variety *Fuji*, where was obtained the lowest content of ash and organic acids.
- In the dried apple fruits, the highest content of total sugars was determined in the variety *Fuji*.
- Dried fruits from the variety *Idared* contained the highest percent of dry matters (94.50 %) and vitamin C (21.12 mg/100g).
- In dried fruits from variety *Golden delicious* was determined the highest content of ash.
- The same content of organic acids was determined in dried fruits from *Golden delicious* and *Fuji* (1.48 % in variant I and 1.32 % in variant II). In dried fruits from variety *Idared* the content of organic acids was higher and its value was 1.81 % (variant II) i.e. (variant I).

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UDC: 637.513.2:614.31(496.5)
Original scientific paper

RISK ASSESSMENT OF POULTRY SLOUGHTERHOUSES IN ALBANIA

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Abstract

The aim of this study is to assess the risk of poultry slaughterhouses in order to achieve a better official inspection. The method adopted in Albania for the categorization of Food Business Operator was based on food products nature. According to this method all slaughterhouse were categorized with medium risk. Through this study we want to compare two methods for categorizing the Food Business Operator base in risk. To this purpose, the study was done in 5 poultry slaughterhouses in Albania. The study was conducted through inspection in establishments to evaluate the characteristics of the plant, product characteristics, production, equipment, implementation of good manufactured practice and good manufactured hygiene, self- control plan (System of Hazard Analysis and Critical Control Points), documentation and analysis of product. In every slaughterhouse, inspections were performed every 3 months, by completing the appropriate checklist using point's evaluation. The results show that 3 of the slaughterhouses were classified with high risk (over 42 points) and 2 of them with medium risk (30-42 points). In conclusion, Food Business Operator can't be categorized based only in one factor which is the nature of food products, but we have to evaluate many other factors.

Keyword: Risk, categorization, slaughterhouse, Food Business Operator.

Introduction

Traditionally, regulatory inspection activity is carried out with emphasis on assessing compliance with all applicable regulations. This kind of inspection is done to improve basic sanitation and to upgrade food establishments. However, with traditional inspection the regulator only tries to find existing food hazards and to ensure their correction. This approach emphasizes reactive rather than preventive measures and it is not focused on preventing future violations from occurring. Developing a risk profile is one of the risk management activities and prioritization of risk would assist in designing and implementing regulatory control measures. A risk-based approach has been suggested as a way of improving the effectiveness of inspection (FAO, 2008). In this modern risk-based approach, the focus of inspection changes from simple compliance verification of a product or food establishment to assessment of the controls put in place in the operation to address food-borne disease risk factors that could put the processor's products at risk.

It is generally not possible for food authorities to inspect all establishments frequently because of factors such as time, cost; lack of human resources; nor is it desirable to inspect

all establishments with the same frequency. The purpose of classifying food businesses according to risk category is to allow the regulatory agency responsible for food inspection to prioritize inspections of food businesses on the basis of the degree of risk they pose to the population so that high-risk food businesses may be inspected more frequently than lower risk food businesses.

EU Food Hygiene Package, and in particular Regulations (EC) No.882/2004 and 854/ 2004, stipulate that official controls in establishments must be carried out on the basis of Risk Categorization of the activities involved and, as well as the type and frequency of official controls must be based on risk assessment.

In Albania, until now, no specific risk assessment audits were carried out in the field. The only source of data indirectly reflecting the risk classification of food/feed establishments is by using the theoretical risk inherent to a particular establishment, calculated as a function of pre-defined assessment criteria. This categorization is used for the implementation of the NFA's Annual Control and Sampling Plan for year 2014. The method adopted in Albania for the categorization of Food Business Operator was based on the types of food that are handled or the processing procedures that are used by food businesses. According to this method all slaughterhouse were categorized with medium risk. But through this study we want to show that the method adopted by NFA's Albania for the categorization of Food Business Operator is not completed. Levels of risk are attributable to a number of factors including:

- Profile factors such as those that consider the type of operation, population served, and the complexity and extent of food handling; and
- Performance factors such as those that are attributable to the operators performance and commitment to food safety practices (i.e., compliance with regulations, commitment to training of food handlers, and the extent to which they incorporate food safety plans into their operations).

Materials and Methods

For this study we analyzed data from NFA, we use different literature and we investigate in field.

To conduct this study are chosen 5 poultry slaughterhouses in Albania.

We used a theoretical risk inherent to a particular establishment, using pre-defined assessment criteria, qualitatively expressed as High, Medium, or Low. The Project CARDS of 2005 produced a comprehensive and documented Technical Guideline for the risk classification of food establishments of animal origin and for setting risk-based priorities of official controls by evaluating 11 criteria grouped in 6 categories, as presented in the table below.

At every slaughterhouse inspections are conducted every three months, filling check list and using point's evaluation.

Basis on risk assessment, poultry slaughterhouse will be grouped in 3 level of risk:

Low Risk (< 30 point) - L

Average Risk (30- 42 points) - M

High Risk (over 42 points) – H

Tabela 1. 11 Criteria for risk classification of food establishments (poultry slaughterhouse)

<i>Category</i>	<i>Category</i>	<i>Low risk</i>	<i>Low/moderated</i>	<i>High/moderated</i>	<i>High</i>	<i>X Factor</i>
1. Characteristics of the establishment	1.1. Date of construction	0	10	20	30	0.10
	1.2. General conditions and maintenance	0	20	40	70	
2. Production	2.1. Size of the establishment and production capacity	0	15	30	50	0.10
	2.2. Size of the market supplied by the establishments	0	15	30	50	
3. Product characteristics	3.1. Type and category of food	0	15	30	50	0.18
	3.2. Intended use	0	15	30	50	
4. Processing hygiene	4.1. Management's expertise and availability to collaborate	0	15	30	50	0.20
	4.2. Training on hygiene and qualifications of personnel	0	15	30	50	
5. Own-checks plan	5.1. Formal thoroughness of the own-checks plan	0	5	15	25	0.22
	5.2. Level of application and adequacy of the plan	0	25	45	75	
6. Past record of compliance	6.1. Irregularities and non-compliances detected in the past	0	30	60	100	0.2

Results and Discussions

The method adopted in Albania for the categorization of Food Business Operator was based on the types of food that are handled or the processing procedures that are used by food businesses. According to this method all slaughterhouse were categorized with medium risk. This categorization is used for the implementation of the NFA's Annual Control and Sampling Plan for year 2014.

The types of food that are handled or the processing procedures that are used by food businesses generally vary from one business to another. Therefore, one food business may have different food-borne disease risk factors from the others. For example, a processing plant producing foods that require cooking prior to consumption such as raw chicken or fish will have different risk factors compared to others that produce ready-to-eat products that do not require cooking prior to consumption. Products such as raw chicken or fish are high-risk foods because they naturally carry a high load of pathogenic bacteria. Therefore, if such products are handled, preparation procedures or practices related to cross-contamination and cooking should be a priority during the inspection. If there are foods that go one or several times through the temperature "danger zone" (4.4–60°C) at which pathogenic micro-organisms are most likely to proliferate, cooling and holding practices must be reviewed (FAO, 2006).

By identifying whether the foods handled or food preparation procedures used are high-risk, the inspector can focus on those foods or procedures that are most likely to cause food-borne diseases if uncontrolled. In addition, the number of expected consumers is another related risk to be considered because a product having a wide distribution and a large number of consumers is more likely to cause extensive outbreaks of food-borne diseases than one with a reduced market reach. Other risk factors include the structure, layout and condition of the premises, type of consumers such as vulnerable segments of the society, e.g. infants/young children, storage, compliance history of the food business and issues such as implementation of food safety management systems, including control systems in place.

Our study was based on evaluation of 11 criteria such as: plant and product characteristics, production, hygiene processes, HACCP, and we achieved the following results (table 1).

Table 2. Evaluation Results for 11 criteria in 5 poultry slaughterhouses

<i>Slaughterhouse Code</i>	<i>05-1</i>	<i>Risk Level</i>	<i>05-2</i>	<i>Risk Level</i>	<i>05-3</i>	<i>Risk Level</i>	<i>11-1</i>	<i>Risk Level</i>	<i>11-2</i>	<i>Risk Level</i>
1. February	72.7	H	45.3	H	71.2	H	68.2	H	49.6	H
2. May	73.2	H	41.5	M	67.0	H	68.2	H	41.8	M
3. September	79.3	H	39.4	M	70.8	H	66.8	H	40.8	M

According to Technical Guideline for the risk classification of food establishments produced by 2005 CARDS Project have been resulted that poultry slaughterhouses are classified as medium risk, but according our evaluation, result that 3 of 5 slaughterhouses involve in this study result with high risk

Conclusions

Classifying food businesses according to risk category is an important step in allowing the regulatory agency responsible for food inspection to prioritize inspections of food businesses on the basis of the potential risk to the population if the safety of food is not ensured.

Official controls in establishments must be carried out on the basis of risk classification of the activities involved and, as well as the type and frequency of official controls must be based on risk assessment. The frequency of inspection should be based primarily on the risk category of the particular food business. Principally, food businesses categorized as higher-risk businesses should be inspected more frequently than those categorized as lower-risk businesses. However, the frequency of inspection may be reduced or increased depending on whether or not compliance and checks are satisfactory during the inspection visit. Some criteria that should be considered for example are risk category of the business, past compliance record, prerequisite activities and application of HACCP.

Risk assessment for each food establishment, should be based on site frequently audit, where is foreseen to be completed the evaluation check list, for 11 criteria defined by point. Laboratory tests should be done in order to verify the accuracy implementation of Good Hygiene-Practices, Good Manufactory Practices and self-control plan (HACCP).

A precise classification of food establishment based on risk will guarantee the conditions for their normal functioning hygienic parameters.

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**PROTEOLYSIS AND LIPOLYSIS DURING RIPENING OF GOAT
WHITE BRINE CHEESE**Srbinovska S.^{1*}, Baltadzieva M.², Santa D.¹¹Faculty of Agricultural Sciences and Food - Skopje, University Ss. "Cyril and Methodius"
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Abstract

White brine cheese is typical for Balkan region, and it has important place in international trading. Traditionally it has been produced from sheep, cow milk or mixed. The sensorial quality and typical flavor of white brine cheese come from degradation of lactose, proteins and fat during ripening period. Dynamic of protein fractions, free amino acids and free fatty acids during ripening period of goat white brine are present in the paper. White brine cheese on 60th day of the ripening contained 47,2±0.39% dry matter, 22,74±0.38% fat, 17,10±0.21% proteins, (0,89±0.058% SN, 0,64±0.047% PN, 0,36±0.039 AAN), 3,60±0.42% salt, 2,52±0.20 lactic acid and pH 4,56±0.02%. The total content of free amino acids and free fatty acids were 176.32±0.71 mg 100g⁻¹ and 0.1173 mg 100g⁻¹, respectively. The principal AA were found to be lysine 33,11± 0,41 mg 100g⁻¹, then leucine 21,57± 0,36 mg 100g⁻¹, phenylalanine 15,61± 0,31 mg 100g⁻¹ and valine 14,80 ±0,16 mg 100g⁻¹, but tyrosine was not detected. From FFA butyric acid was dominant (0.0421 mg 100g⁻¹). In goat white brined cheese during the ripening slow lactic acid fermentation is present, which only allows the creation of acetic and propionic acid that give sour taste and also butyric acid which affects the creation of specific goat flavor.

Introduction

The specific goat milk composition is suitable for technological processes which are characteristic for brined cheeses, especially on white brine cheese. Ability of goat proteins to retain water, specific structure and rheological properties of goat curd enable optimal regulation of fermentative process in cheese. The specific fatty acid composition and characteristics of fat globules (Mehia, 1995) and also triglycerides' structure (Gall, 1981) mostly determine plastic, porous texture of the curd, specific flavour of final product. Due to their specific composition, organoleptic characteristics and healthy attributes, the production of goat's milk and goat cheese has attracted growing interest over recent years (Silanikove *et al.*, 2010).

White brined cheese is characterized with specific fermentation process which mainly takes place in brine, as well as with the characteristic diffusion of salt in the cheese. Brine concentration had the most negative effect on WSN and TCA-SN formation, but also high concentration of NaCl in this cheese inhibits proteolysis (Guinee and Fox 2004 Alizadeh *et al.*, 2006). In brine cheese, unlike other types semihard cheeses, proteolysis is limited,

and the hydrolysis of fat is undesirable. Therefore, from the sensory properties, lactic acid taste and aroma dominate (Kosikowski, 1982). Goat milk contributes to the enrichment of some picant taste of cheese due to the specific level of degradation of protein and fats (Attaie and Richter., 1996).

Materials and methods

The goat brine cheese was manufactured for this study according to the method used in macedonian cheesemaking plants. The average milk composition of goat milk used as raw material was as follows: fat $3,84 \pm 0,360$ %, protein $3,21 \pm 0,034$ %, casein $2,49 \pm 0,031$ %, lactose $4,49 \pm 0,077$ %, minerals $0,75 \pm 0,027$ %, with pH acidity $6,65 \pm 0,056$. Milk was pasteurized at 72°C for 20 s, and cooled to $32\text{--}34^{\circ}\text{C}$, added of $0,015$ %, CaCl_2 by the addition of $0,3$ % starter culture (*St. lactis*, *Lb. casei*) 30 min before renneting. After coagulation (60 min), the curd was cut and then 3 times stirred and the whey drained off when the pH of whey was 6.4-6.5. The curd was pressed with 5 kg weights on 1 kg final curd, then cut to a suitable shape and size (10x 10x 7 cm) and soaked in a brine (20-22%, w/v) for 10-12 h on 15°C . On the next day the cheese pieces were placed in tins, brine (10%, w/v,) and transferred to a ripening room at $10\text{--}12^{\circ}\text{C}$ in the period of 60 days, after which the tins were placed in a cold room at 5°C .

The following methods were used to determine the composition of the cheese: dry matter (IDF, 1982), fat (EN ISO, 2008b), salt (Mohr's method, Pejić and Đorđević, 1963), titratable acidity (Carić et al., 2000), pH (Mettler Toledo), total nitrogen (TN) (Kjeldahl method (IDF, 1993) and nitrogen fractions (Pejić and Đorđević, 1963), free amino acids method Martini Seidzheim (Inihov, 1971) using the automated aminoanalyzer HD-1200; and free volatile fatty acids - method Demurov, 1976 using a gas chromatograph (Model, Varian vista 6000, USA, FID detector, DEGS column).

The research was done on proteolyses and lipolyses of white brine goat cheese for periods of 5, 10, 20, 40 and 60 days of ripening using traditional technology, with 4 replications.

Results and Discussion

Dinamics of main components in goat white brined cheese during the maturation are presented in Table 1. The moisture content in the white brined cheese during ripening period of 60 days, gradually decreased and on the 5th day was $58,8 \pm 1,61\%$, on the 20th day $56,2 \pm 2,7\%$ and at the end of the process reached to $52,8 \pm 2,39\%$.

Lactic acid continuously was increasing, from $1,89\%$ on the 5th day, $2,16\%$ on the 20th day and at the end of maturation reached $2,52\%$.

The pH is much lower than in other types of cheese and in the ripening process decreases, on the 5th day it was $4,9 \pm 0,07$, the 40th day was $4,6 \pm 0,02$ and then by the end of maturation remained almost unchanged. In the cheeses with higher titratable acidity the LAB activity is slower, so creating endoenzymes is less leading to a reduced concentration of puffing substances, and hence continually decreasing the pH acidity.

In white brined cheese lactose, glucose and galactose retained longer, up to 60-day due to the low acidity of the brine and higher concentrations of salt. These two factors strongly reduce the activity of lactic acid bacteria and also enzymatic activity (Baltadzieva, 1985; Gruev 1995). Similar results were reported by Baltadzieva (1995) and Kalantzopoulos

(1993) in the study of white brined cheese and feta made from goat milk except that they determined lower titratable acidity at the end of ripening and for 5 °T or 10 °T accordingly. Galiou et al. (2015), who investigate the chemical characteristics of traditional goat cheeses from Morocco on the 5th day of ripening found lower concentration of fat and protein content. This difference probably is due to the different technological process and different composition of goat milk. Smiljanic et al (2014) reported low concentrations of moisture, proteins in dry matter and fat in dry matter of a white brined cheese from row cow's milk. Continually decreaseing of pH was reported by Barac et al (2013) on the white brined goat cheese where pH on the 60th day of ripening reached 4,20, while the moisture was significantly higher compared with our results.

Table 1. Goat white brine cheese composition

Parameters	Ripening period (days)				
	5	10	20	40	60
Moisture %	58,8±0,61	57,2±0,54	56,2±0,47	54,4±0,80	52,8±0,39
Dry matter %	41,2±0,61	42,2±0,54	43,8±0,47	45,6±0,80	47,2±0,39
Acidity °T	210±0,8	224±0,9	240±1,0	260±2,3	280±1,2
Lactic acid %	1,89±0,09	2,02±0,11	2,16±0,17	2,34±0,19	2,52±0,20
pH	4,90±0,07	4,81±0,04	4,70±0,06	4,60±0,02	4,56±0,02
Fat %	22,17±0,26	21,98±0,23	22,35±0,41	22,60±0,25	22,74±0,38
FDM %	53,81±1,29	52,08±1,82	51,03±1,90	49,56±2,02	48,18±1,74
Proteins %	17,48±0,78	17,86±0,92	17,22±0,72	17,73±0,93	17,10±0,84
Proteins in DM%	42,43±1,03	42,32±1,34	39,31±0,98	38,88±1,12	35,23±0,98
Salt %	3,40±0,51	3,48±0,04	3,55±0,16	3,58±0,35	3,60±0,42
Salt in moisture %	5,78±0,91	6,08±1,05	6,31±1,35	6,58±0,76	6,80±0,98

Total salt content ranged between 3,40±0,51 at the 5th day up to 3,60±0,42 g 100g⁻¹ at the 60th day and salt in moisture in the end of the ripening was 6,8 g 100g⁻¹, which is in correlation with standards for feta cheese where the minimum SM value should be 4,3 g 100 g⁻¹.

According the results can be conclude that the lactic acid process in white brined cheese from goat milk is closer to the cheese made from sheep milk which was also confirmed by Kalantzopoulos (1993). Specific mineral composition of goat milk influence on higher lactic fermentation in cheese (Mahien et al, 1977; Stordz et al, 1983; Jaurez et al., 1985). These properties in general explain the gradual changes in the moisture content at the time of ripening

The content of water soluble nitrogen (WSN) increases during the ripening of the white brined cheese and on the 5th day was 0,60 ± 0,046%, on the 20th was 0,68 ± 0,048% and the 60th day reached to 0,89 ± 0,058%, which is 1,48 times more than the day 5 (table 2.). The content of WSN in the TN ranged from 21,89% on the 5th day up to 33,21% on the 60th day of ripening.

Peptides nitrogen (PN) gradually increased during ripening. It takes most of the WSN and the 5th day was 70,00%, and at the end of maturation 71,91% from WSN. Participation of PN in the TN is 15,32% on the 5th day and in the end of the ripening increased to 23,88%.

Table 2. Nitrogen fractions in goat white brined cheese (%)

Parameters	Ripening period (days)				
	5	10	20	40	60
Total N,	2,74 ± 0,48	2,80 ± 0,32	2,70 ± 0,27	2,78 ± 0,43	2,68 ± 0,52
Water Soluble N,	0,60±0,046	0,63±0,059	0,68±,048	0,76±0,062	0,89±0,058
Peptides N,	0,42±0,038	0,48±0,064	0,52±0,058	0,58±0,064	0,64±0,047
Amino acid N,	0,13±0,048	0,14±0,053	0,21±0,060	0,25±0,062	0,36±0,039

The content of Amino acid nitrogen (AAN) also increased in the ripening process and the 5th day was 0,13 ± 0,048, at 20th day increased for 1,62 times in the 60th day for 2,77 times. The percentage of presence of AAN related to WSN at the end of maturation was 40,49%, and the presence of the total AAN at the end of maturation was 13,43%. Similar results for the total nitrogen content were reported by Atasoy and Turkoglu (2008) on Urfa cheese which is a white brined Turkish cheese.

Participation of thermophilic lactic acid bacteria in the ripening of white brined cheese is minimal because of high salt concentration and low ripening temperature.. This cheese is characterized by slower degradation of paracasein due to the relatively low pH (Gruev, 1995). Proteins during ripening are transformed into different products from which mainly influence on the organoleptic quality of the cheese. Particularly important is water soluble nitrogen (WSN) because affect to the curd properties, its aroma, taste and smell. Based on soluble nitrogenous substances can be estimated the intensity of protolysis during the cheese ripening.

The proteolysis is characterized by the amount of WSN (0,89±0,06%), so the maturity degree of final goat brined cheese was 33,21%, which is in correlation with other brined cheese. The cheese undergoes extensive proteolysis and lipolysis during the maturation period, and maturity degree according the ripening periods was very high in the beginning, due to largest increase of soluble nitrogen in the same period, probably because of the appropriate conditions for LAB development in early days of cheese ripening.

During the cheese ripening free amino acids are increasing. Different types of cheeses are distinguished by the amount and the quantitative ratio of free amino acids, which depends of the type of milk, milk heat treatment, rennet activity, type of starter culture, curd heating, maturation and other technological procesis. The release of free amino acids (FAA) in brined cheese was investigated at various stages of ripening.

The concentration of FAA increased almost twice throughout ripening, after 60 days compared with day 5 (Table 3). Their presence in cheese is relatively low which depends by the specific fermentative process in cheese. The principal AA were found to be lysine 33,11± 0,41 mg 100g⁻¹, than leucine 21,57± 0,36 mg 100g⁻¹, phenylalanine 15,61± 0,31 mg 100g⁻¹ and valine 14,80 ±0,16 mg 100g⁻¹. The least present were threonine (3,71 ± 0,62 mg 100g⁻¹) and proline (3,61 ± 0,33 mg 100g⁻¹) and tyrosine was not detected. Total amount of free aminoacids on the 15th day of ripening was 89,18±0,56 mg 100g⁻¹, on 30th day was 134,13±0,64 mg 100g⁻¹, and on the end of ripening process it reached to 176,32±0,71 mg 100g⁻¹, which is for 1,98 times higher than at the beginning of ripening process.

Table 3. Free amino acids in goat white brine cheese

Free amino acid mg 100 g ⁻¹	Ripening period (days)		
	15	30	60
Lysine	16,20 ± 0,31	21,40 ± 0,62	33,11± 0,41
Histidine	2,20 ± 0,13	7,41 ± 0,36	16,12 ± 0,71
Arginine	2,70 ± 0,71	4,17 ± 0,62	4,85 ± 0,27
Threonine	2,60 ± 0,78	3,18 ± 0,16	3,71 ± 0,62
Valine	11,78 ± 0,61	13,18 ± 0,17	14,80 ± 0,16
Methionine	6,11 ± 0,43	8,17 ± 0,27	8,71 ± 0,62
Isoleucine	3,21 ± 0,62	4,15 ± 0,15	4,52 ± 0,27
Leucine	11,72 ± 0,31	18,28 ± 0,36	21,57 ± 0,36
Phenilalanine	4,11 ± 0,62	7,12 ± 0,28	15,61 ± 0,31
Aspartic acid	3,80 ± 0,11	8,12 ± 0,48	9,11 ± 0,37
Serine	6,10 ± 0,18	10,21± 0,61	12,11± 0,38
Glutamic acid	6,70 ± 0,79	7,80 ± 0,22	9,28 ± 0,65
Proline	1,50 ± 0,28	2,91 ± 0,68	3,61 ± 0,33
Glycine	/	2,11 ± 0,63	6,63 ± 0,58
Alanine	3,31 ± 0,41	3,81 ± 0,12	4,11 ± 0,43
Cystine	4,81 ± 0,11	6,28 ± 0,31	7,51 ± 0,38
Tyrosine	/	/	/
Total content	89,18 ± 0,58	134,13±0,64	176,32±0,71

The characteristic absence of the amino acid tyrosine is in agreement with the results by different authors which found it in very low concentrations or they do not detect in the goat cheeses (Veinoglou et al., 1982; Baltadjieva, 1985; Stamenova, 1985).

Comparing the content of free amino acids in goat cheese with the similar cheeses from cow's milk or sheep's milk, it can be concluded that goat cheeses contain significantly higher amounts, as it was confirmed by other authors (Litopoloui-Tsantaki and Manolkidis, 1985; Baltadzieva 1985), probably due to higher enzyme activity in goat milk (Khatoon et al., 1990). Concentration of free fatty acids (FFA) in goat brine cheese at various stages of ripening was investigated (Table 4.) It was determined low concentration of acetic (0.0231 mg 100g⁻¹), propionic (0,0121 mg 100g⁻¹) and butyric acid (0,0314 mg 100g⁻¹) which amount is slightly changed in the period from the 5th to the 60th day, probably due to the degradation of protein and lactose, and not from the lipolysis (Gruev, 1995). The amount of acetic acid at the end of maturation was increased to 1,4 times compared to the 10th day, propionic to 3,4 times and butyric for 1,34 times. This organic acid is produced in the cheese as a result of citrate and lactose fermentation, or from amino acid catabolism, or from lipolytic activity (McSweeney and Sousa, 2000). Acetic and propionic acid can be created by the fermentation of lactose and lactic acid (Gruev 1995, Eck 1986, Scott, 1986), therefore they are mainly present in white brined cheese.

Table 4. Free fatty acids in goat white brine cheese

Free fatty acids mg 100g ⁻¹	Ripening period (days)		
	10	30	60
Acetic acid	0,0231	0,0252	0,0331
Propionic acid	0,0121	0,0301	0,0421
Butyric acid	0,0314	0,0328	0,0421

The concentration of butyric acid increased during the cheese ripening but mostly over 50% of the total was created at the beginning of ripening (74,58%). Lower degree of butyric acid creation during ripening, especially at the end of the ripening of the cheese, probably is due to the lack of free substrate conversion into fatty acids by lipolysis or reduction of enzymatic activity due to change in the microstructure of the cheeses. According Attaie and Richter (1996) butyric acid has great influence on the taste of the cheese.

In goat white brined cheese during the ripening slow lactic acid fermentation is present, which only allows the creation of acetic and propionic acid that give sour taste and also butyric acid which affects the creation of specific goat flavor. Other free fatty acids in brined cheese from goat's milk are not detected in our study and the results are similar with those by Woo et al., (1984) and Law (1982).

Conclusion

White brined cheese from goat milk is characterized with appropriate proteolysis - typical for brined cheeses. This cheese in structure and flavor is more similar with the sheep brine cheese according the lactic processes, dynamics of main components, proteolyses and lipolyses during cheese maturation.

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ANTIFUNGAL EFFECT OF SODIUM BENZOATE AND POTASSIUM SORBATE ON *PENICILLIUM* SP. IN *in vitro* AND *in situ* CONDITIONSManić V.¹, Gradvol V.¹, Pavlović H.*¹Faculty of Food Technology Osijek, Croatia

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Abstract

The aim of the study was to determine antifungal activity of sodium benzoate and potassium sorbate on selected fungal species: *Penicillium aurantiogriseum*, *P. expansum* and *P. italicum* at different pH values. Sodium benzoate is a most widely used preservative in carbonated drinks, fruit juices, jams, conserved vegetables, pickles and condiments where, at lower pH environment, inhibits fungal contaminants. Potassium sorbate is primarily used as a food preservative. It inhibits molds and yeasts in many food products, such as cheese, wine, yogurt, soft drinks and baked goods. Important effect of potassium sorbate is to maintain and prolong quality of food products. The experimental results suggest increased antifungal activity of tested compounds at lower pH values. Both compounds inhibit fungi at similar concentrations. Linear inhibition of fungal colony growth at 50, 100 and 200 ppm of Na – benzoate and K – sorbate at pH 5,12 and 4,50 has been tested. Preliminary results suggest stronger inhibitory potential of K – sorbate, compared to Na – benzoate

Key words: K-sorbate, Na-benzoate, *Penicillium*, inhibition, pH value

Introduction

Fungi are very important group of microorganisms in food processing and preservation. Contrary to bacteria, fungi are capable to grow at lower a_w , as well as pH. In favourable food/conditions, their pervasive growth makes deterioration control difficult.

Two genera of molds stand out considering economic importance in food spoilage: *Penicillium* and *Aspergillus* (Pitt and Hocking, 2009).

Most *Penicillium* species are ubiquitous, opportunistic saprophytes, being able to grow in almost any environment and in wide range of physical and chemical environments: a_w , pH, temperature, pH and redox potential. Although cereal grains appear to be their natural habitat, *Penicillium* sp. are found and cause spoilage of different foods in different environmental conditions. *Penicillium aurantiogriseum* is among most commonly encountered fungi on earth. It is found on cereals, nuts, stored fruits and vegetables, cold stored eggs, etc. It can grow from -2 to 30°C, $a_w > 0,81$ and the growth is stimulated by 10 % of CO₂. *P. expansum* is one of the oldest described *Penicillium* species, established as the principal cause of spoilage of pome fruits. Like many other *Penicillium* species, it is psychrophile capable to grow from -6° to 35°C, a_w minimum 0,82 and very low requirement for oxygen. *P. italicum* is primary spoilage agent of citrus fruits (capable for

growth from 1,6 to 9,8 pH) and requires higher a_w for minimal growth (0,87) (Pitt and Hocking, 2009).

Mold growth in foods can be controlled by numerous methods and procedures like preventing spores from entering foods, adjusting storage temperatures and humidity, altering oxygen levels, using chemical preservatives and related measures. Unfortunately, molds are capable to adjust and stated methods do not prevent molds from altering food quality and safety in every situation in food processing and storage (Jay, 2000).

Chemical preservatives are substances capable of inhibiting, retarding or arresting the growth of micro-organisms or of any deterioration resulting from their presence or of masking the evidence of any such deterioration. Most widespread preservatives in fungal inhibition are sodium benzoate and potassium sorbate (Spadoni et al., 2015). Used in different foods, they inhibit fungal growth in foods especially at lower pH. Preservatives can be used with lower pH values, lower temperature or combined with different chemicals like ethanol (Karabulut et al., 2005) to reduce fungal spores viability. Preservatives have important antimicrobial purpose in food processing, although can be used in post-harvest treatment as well in order to substitute toxic fungicides (Montesinos-Herrero et al., 2009). The aim of this study was to: (1) determine minimal fungicidal (MFC) and inhibitory (MIC) concentration of sodium benzoate and potassium sorbate on three *Penicillium* species at two pH (2) evaluate colony linear growth inhibition at two pH and (3) to test effect of preservatives in *in situ* conditions on apple slices.

Materials and methods

Microorganisms and culture conditions

Fungal species: *Penicillium aurantiogriseum*, *P. expansum* and *P. italicum* were obtained from Charles University Culture Collection in Prague. Cultures were maintained on Potato dextrose agar (Liofilchem, RosetoDegli Abruzzi, Italy) at 4°C. Fungal spores were harvested after 5 day incubation at 25°C in 0,2 % agar with 0,05 % Tween 80 (Biolife, Milan, Italy) and adjusted to 1×10^6 spores/mL by haemocytometer.

Minimal fungicidal and minimal inhibitory concentration

To determine minimal fungicidal (MFC) and minimal inhibitory (MIC) concentration macrobroth dilution method (Agarwalet al., 2000) was applied. Preservatives (K-sorbate and Na-benzoate; Kemika, Zagreb, Croatia) were tested by macrobroth two-fold serial dilution technique. Preservatives were dissolved in water to get a stock (300 mg/mL) solution, filter sterilized and dilutions were made serially. Fungal spores were inoculated (10^6 CFU/mL) in Potato dextrose broth (Liofilchem, Rosetodegli Abruzzi, Italy) with pH adjusted to 5,12 and 4,5 by 1 M HCl. The tubes were incubated at $25 \pm 1^\circ\text{C}$ and the MFC and MIC (in ppm) was recorded after 72 h of incubation. Suitable controls: broth control (without fungal spores) and growth controls (with fungal spores) were set under identical conditions. The last tube with no apparent growth of the organism represented the MIC of the compound.

Colony growth inhibition

Inhibition of radial colony growth was tested in Petri plates on Potato dextrose agar (Liofilchem, Rosetodegli Abruzzi, Italy) amended with preservatives with pH adjusted to 5,12 or 4,5. After solidification, plated were inoculated in the centre with 2 μL of spore

suspension with concentration of 1×10^6 CFU/mL. Plates were prepared in triplicate and incubated at 25°C. In controls, preservatives were omitted. During incubation at 25°C the radial growth of the fungal colony was measured every 2 days in two directions. For each culture the radial extension of colonies were plotted against time. Daily measured radii were analyzed statistically by analysis of variance (ANOVA) ($P < 0,05$) followed by the Bonferroni post hoc multiple comparisons by XLSTAT Version 2009.3.02 (Addinsoft SARL, 1995-2009). In all cases the experiments were carried out in triplicates per treatment. All treatments were repeated at least two times.

In situ experiment

For *in situ* conditions, apple slices (Idared) were used. After washing in tap water, sterile demineralized water and whipped of with 70 % ethanol. Apples were cut with sterile knife to slices of 5 mm thickness and cored to 22 mm round slices by sterile metal cutter. Apple slices were dipped for 30 seconds in 2 % ascorbic acid solution (to prevent browning) and drained on sterile paper towels in laminar hood under UV. After drying, slices were dipped in preservative solution (50, 100 and 200 ppm) for 30 seconds. After draining on sterile paper towels, 4 apple slices were transferred to Petri dish in two repetitions. Each slice was inoculated with 2 $\square$ L suspension of fungal spores (1×10^6 CFU/mL) and incubated at 25°C for 6 days. Radius of fungal colony was measured every other day in two perpendicular directions.

Results and discussion

Chemical compounds or preservatives with low toxicity and generally recognized as safe (GRAS) compounds (like K-sorbate and Na-benzoate) are amongst most important preservatives in food processing and have received increasing interest for the control of postharvest fruit disease like blue mold apple decay or green mold or sour rot of citrus fruits (Spadoni et al., 2015).

Antifungal activity of tested preservatives to selected *Penicillium* species (Table 1) was very uniform at both tested pH values (5,12 and 4,5). Seems that difference in pH units was not enough to show difference in inhibition or fungicidal action in this experimental section. Manso et al. (2015) have noticed that pH value lowering from 7 to 5 and 3 have noticeable effect in lowering concentrations of cinnamon and oregano essential oils MIC and MFC. It is worth to mention that incubation time in this part is relatively short (3 days), and fungi are transferred to sterile PDA broth without adjustment pH adjustment in order to establish MIC or MFC activity. This transfer allows fungi to repair damages in cells so fungi, even grown at lower pH can recuperate. Both *P. expansum* and *P. italicum* showed sensitivity to K-sorbate (200 ppm MIC, > 800 ppm MFC) while to inhibit *P. aurantiogriseum* double concentration of K-sorbate was required. K-sorbate exhibited fungicidal activity in concentration more than 800 ppm, to all tested species. Contrary to K-sorbate, *P. aurantiogriseum* was the most sensitive fungi to Na-benzoate, with 200 ppm (at both pH value tested) for MIC concentration. Although similar in morphology even members of the same subgenus *Penicillium*. Compared to K-sorbate, Na-benzoate increases it's toxic effect at lower pH (MFC of *P. aurantiogriseum* and *P. expansum* is 800 ppm). In this experimental setup it is evident that by lowering pH value, fungal growth is retarded. Membré et al. (2001) have found that sorbic and propionic acid also have a significant inhibitory influence on the growth rate of *P. brevicompactum* since they led to a

50% reduction of mycelium proliferation at pH 7 and greatly inhibited the mould at pH 5. On the other hand, Na-benzoate did not significantly modify the growth rate, as compared to the control. This result mostly supports our findings where Na-benzoate had reduced potency in retarding tested fungi growth, compared to K-sorbate. Similarly, Lopez-Malo et al. (2007) have reported lower MIC of Na-benzoate at 3,5 (400 ppm), compared to experiment performed at pH 4,5 on *Aspergillus flavus*. Benzoate has a pK value of 4,20 and since the antimicrobial activity of benzoate resides in the undissociated form, therefore, weak acid preservatives exist in a pH dependent equilibrium between the undissociated and dissociated state. Optimal inhibitory activity takes place at low pH since it favors the undissociated state of the molecule that is freely permeable across the plasma membrane. Once the neutral molecule enters the cell it will dissociate due to a higher pH. The preservative action is thought to be due to an accumulation of anions and protons inside the cell (Bruland & Coote, 1999; Jay, 2000).

Table 1. Effect of K sorbate and Na benzoate on selected *Penicillium* species

Species	Effect	K-sorbate		Na-benzoate	
		pH 5.12	pH 4.5	pH 5.12	pH 4.5
<i>P. aurantiogriseum</i>	MIC*	400 ***	400	200	200
	MFC**	> 800	> 800	> 800	800
<i>P. expansum</i>	MIC	200	200	800	800
	MFC	> 800	> 800	> 800	800
<i>P. italicum</i>	MIC	200	200	800	800
	MFC	> 800	> 800	> 800	> 800

* = minimal inhibitory concentration

** = minimal fungicidal concentration

*** = values expressed in ppm

As mentioned, contact with preservatives during MIC and MFC determination was relatively brief (48 h of incubation in broth with chemicals, additional 24 h in pure broth for confirmation). Preservatives inhibition spore germination, germ-tube elongation as well as enzyme activity in cytoplasm (Hervieux et al., 2002) resulting in postponed or retarded growth of fungi. This effect is more pronounced in retarded fungal colony growth in a growth medium amended with K-sorbate, especially at lower pH (4,5) (Fig 1-3). During 18 day of incubation, Na-benzoate acts similarly regarding difference in pH, although it is worth to mention that 200 ppm at final incubation period is significantly different compared to other samples (Fig 1). The situation with K-sorbate is different. Even at pH 5,12, growth at 100 and 200 ppm showed significant difference, more pronounced at lower pH where all concentrations applied were significantly different, compared to control sample. Additionally, lag phase of fungal growth is prolonged to 3 days. This can be important during storage of perishable fruits or ready to eat fruit salads.

Although *P. expansum* is a notorious for a rapid growth on storage fruits, in our experimental setup was the slowest growing specie (Fig 2). The reason could be more nutrition elements in real substrate (fruit), compared to growth media. Na-benzoate affects this specie growth, especially at lower pH, higher concentration with more potency, but the differences in colony radii were not statistically significant, although the difference exist. K-sorbate was proved to be more effective in colony growth inhibition, with statistically significant difference at 4,5 although uniform effect is noticeable even at 5,12. Effect of

higher concentration is visible after 10th day, although no significant difference was detected.

P. italicum was the most rapid growing specie of *Penicillium* genera tested in our experiment (Fig 3). It reached the end of 9 cm Petri dish in 12 days (Fig 3). As presented in Fig 3, Na-benzoate had no effect during experiment, although some differences in colony size are obvious at lower pH, but there is no statistical significance. K-sorbate fungal inhibit was stronger than Na-benzoate, at both pH values. Although no statistical difference was observed at pH 5,12, all concentrations of preservative applied slowed fungal growth, nevertheless, at 14 day of incubation period, all samples reached the end of the dish. Retarding of fungal growth was more potent at pH 4,5 where lag phase of 2 days was observed, as well as statistically significant difference in the inhibition at highest concentration of Na-benzoate applied (200 ppm). At the highest inhibition moment, colony radii difference is more than 20 mm.

To test *in situ* effect of preservatives, Idared apples was used. To avoid rapid overgrowth of fungi, *P. expansum* was tested with Na-benzoate, more common preservative. The effect of Na-benzoate was more pronounced to fungal colony growth inhibition (Fig 4), compared to the results of colony growth on PDA (Fig 2) implying the need for testing antimicrobial effect in real systems, as well as growth media. Na-benzoate of 200 ppm was the only one that retarded fungal overgrowth even on 6th day of incubation period at 25°C. The least effective preservative was K-sorbate at lowest concentration (50 ppm). Incubation temperature throughout the experiment was uniform (25°C) to maximize favourable environment for fungal growth. Lowering temperature to 4-8°C we can assume more pronounced, synergistic effect of lower temperature and preservative is to be expected.

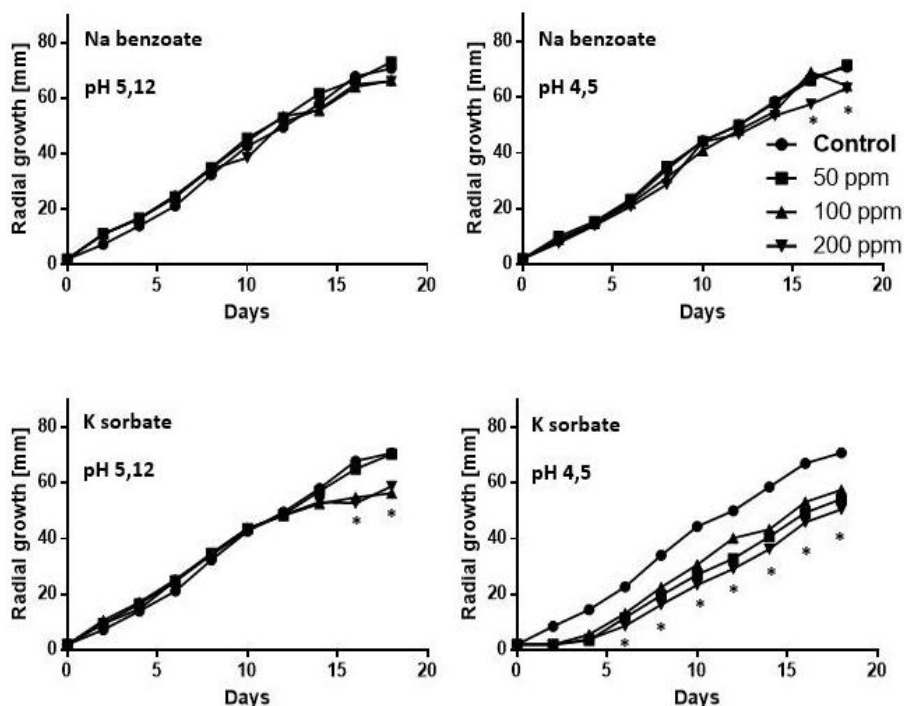


Figure 1. Effect of preservatives on *P. aurantiogriseum* radial colony growth at pH 5,12 and 4,5
* = significantly different ($p < 0,05$) according to Bonferroni multiple comparisons

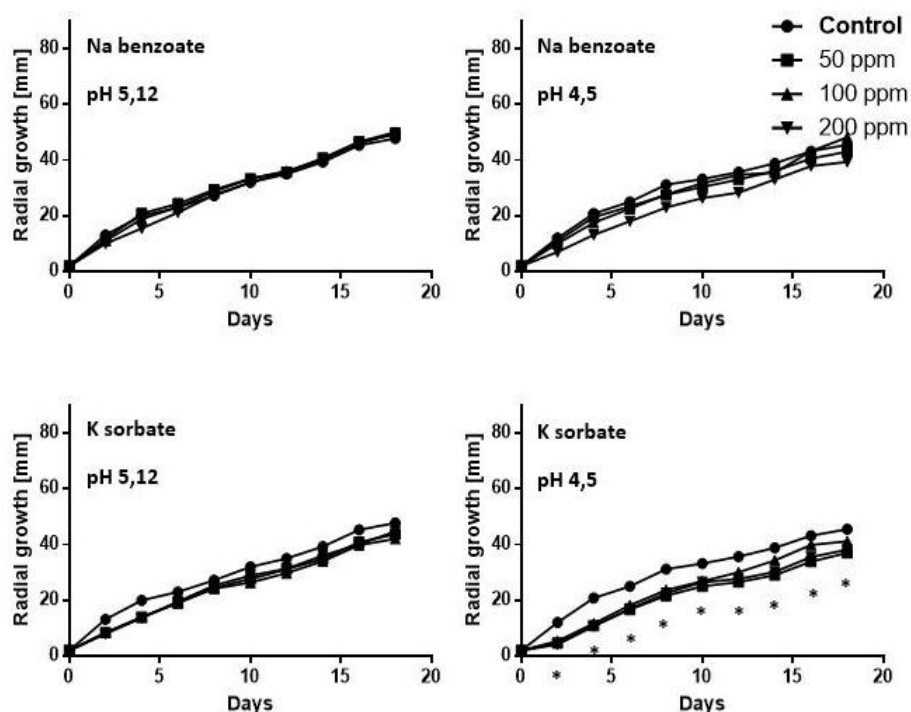


Figure 2. Effect of preservatives on *P. expansum* radial colony growth at pH 5,12 and 4,5
* = significantly different ($p < 0,05$) according to Bonferroni multiple comparisons

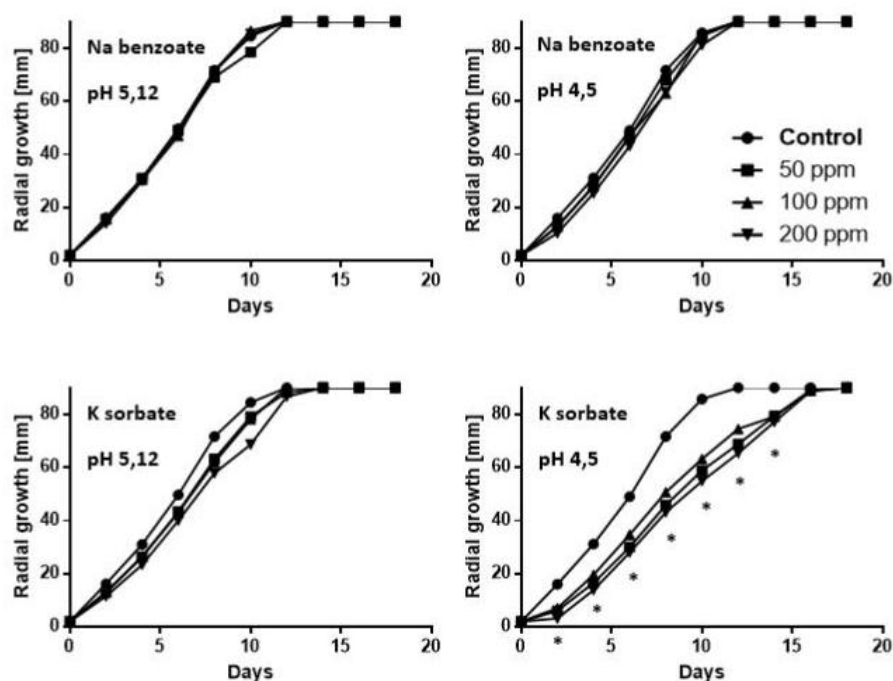


Figure 3. Effect of preservatives on *P. italicum* radial colony growth at pH 5,12 and 4,5
* = significantly different ($p < 0,05$) according to Bonferroni multiple comparisons

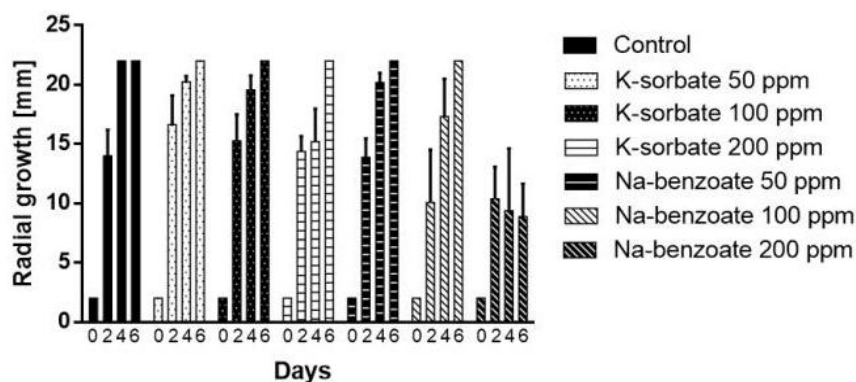


Figure 4. Effect of preservatives on *P. expansum* during incubation on apple slices (error bars represent SD of 8 results)

Conclusion

Usage of preservatives in food processing is still important in different food production despite negative perception by some consumers. Obtained results suggest the need different species of test microorganisms in experimental usage since their different response to chemical applied. Preservatives activity is, usually concentration dependent so stronger inhibition is expected by higher concentration applied. Since difference in composition compared to growth media, real food systems must be included in testing preservative efficacy.

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UDC: 613.262-053.6(497.6)
Original scientific paper

REPRESENTATION OF FRUITS AND VEGETABLES IN ADOLESCENTS' DIET IN CANTON SARAJEVO, BOSNIA AND HERZEGOVINA

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Abstract

Adolescents are the population that according to numerous studies do not consume a sufficient amount of fruits and vegetables which are rich in vitamins and minerals, specially needed in this period of life. Therefore, the aim of the research was evaluation of representation of fruits and vegetables in the diet of adolescents living in Canton Sarajevo that are observed with regard to sex and place of residence. The study included 630 adolescents. Statistical significance was demonstrated only regarding male adolescents for having/not having the habit of consuming fruits and vegetables ($\chi^2=4.079$, $p=0.043$). Nevertheless, adolescents of both sexes and in both areas consume more fruit than vegetables, but also fruit is not present in a satisfactory amount.

Key words: adolescents, diet, fruits, vegetables.

Introduction

Adolescents, a population between 10-19 years (WHO, 2005; UNICEF, 2012), according to numerous studies (Hodžić&Smajić, 2012; Bertić, 2013) do not consume a sufficient amount of fruits and vegetables which are rich in vitamins, minerals, dietary fiber and complex carbohydrates. Those are needed for physical and psychological growth and development. This research has been conducted in order to know the situation regarding consumption of fruits and vegetables among adolescents living in Canton Sarajevo.

Material and methods

Research involved 630 adolescents, 133 from rural area (60 males and 73 females) and 497 adolescents from urban area of Canton Sarajevo (264 males and 233 females). Division of participants according to the place of residence was done upon Spatial plan of Canton Sarajevo (Zavod za planiranje i razvoj Kantona Sarajevo, 2006). Distribution of participants was in accordance with demographic data of Institute for Statistics of F B&H for Canton Sarajevo (Federalni zavod za statistiku, 2013). Participants were aged 13-15 years. The survey was conducted in the schools with the approval of the Ministry for education, science and young of Canton Sarajevo. As an instrument was used purpose-designed questionnaire. The students filled it in in the presence of the author and the teacher. Statistical analysis was done with SPSS 13.0.

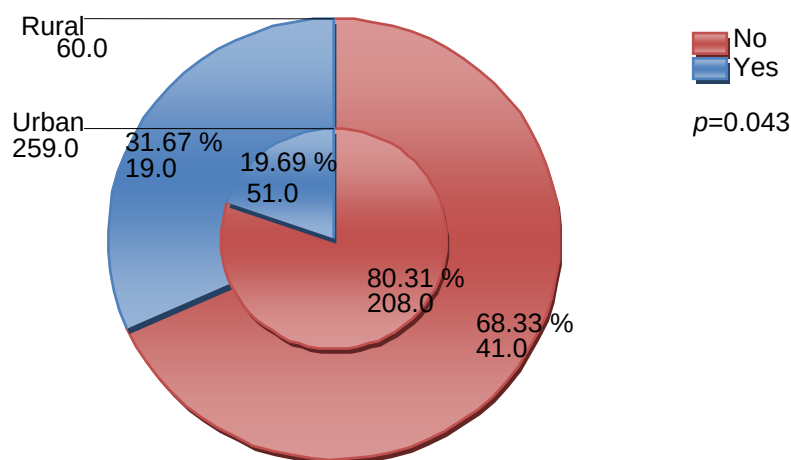
Results and discussion

Results are presented by sex and the place of residence. It is known that with sufficient energy supply, even smaller deficits of protective nutrients that are not associated with clinical symptoms, especially if they persist for a long time, can adversely affect the health and functional state of the organism. This is primarily related to the growth and development of children, physical ability, the functioning of the immune system, as well as the mental functions (Gibney et al., 2002).

Table 1. Habit of consuming fruits and vegetables among female adolescents

			Do you eat fruits and vegetables every day		Total
			No	Yes	
Place of Residence	Rural	N	61	11	72
		%	84.70%	15.30%	100.00%
	Urban	N	183	48	231
		%	79.20%	20.80%	100.00%
Total		N	244	59	303
		%	80.50%	19.50%	100.00%

The habit of eating fruits and vegetables and place of residence of female adolescents are not correlated with one another ($p=0.059$, $p=0.305$). Extremely high percentage of adolescents do not consume fruits and vegetables every day (rural vs. urban: 84.70 %: 79.20 %).



Graph 1. Habit of consuming fruits and vegetables among male adolescents

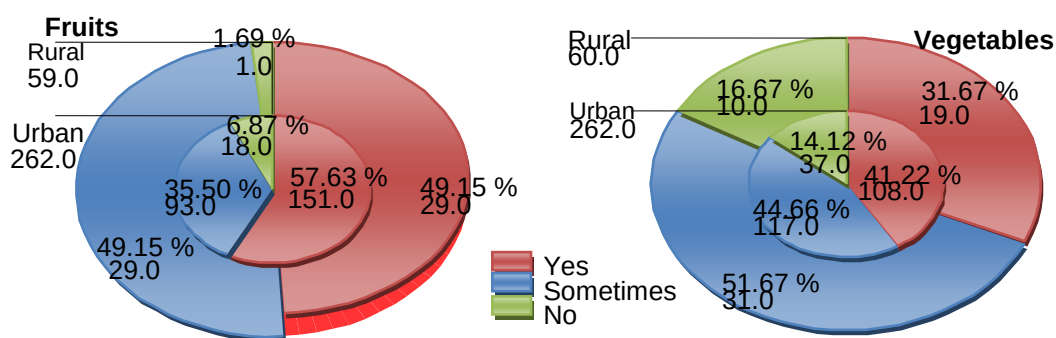
The habit of consuming fruits and vegetables depends on the place of residence of male adolescents ($\chi^2=4.079$, $p=0.043$). Very high percentage of urban adolescents (80.31 %) do not consume fruits and vegetables daily. It is lower percentage of rural adolescents who do not have a habit of consuming it (68.33 %) regularly.

SECTION 4. FOOD QUALITY AND SAFETY

Table 2. Consumption of fruits and vegetables per portion among female adolescents according to the place of residence

	Place of Residence		Do you eat at least 2 portions daily?			Total
			Yes	Sometimes	No	
Fruits	Rural	N	39	29	5	73
		%	53.40	39.70	6.80	100.00
	Urban	N	124	98	11	233
		%	53.20	42.10	4.70	100.00
Vegetables	Rural	N	24	41	8	73
		%	32.90	56.20	11.00	100.00
	Urban	N	94	108	30	232
		%	40.50	46.60	12.90	100.00

Recommended amounts of fruits and vegetables were used as presented in the charts of USDA's latest guidelines (<http://www.choosemyplate.gov/food-groups/fruits-amount.html>, <http://www.choosemyplate.gov/food-groups/vegetables-amount.html>). Fruit consumption per portion does not depend on the place of residence of female adolescents ($\rho = -0.007$, $p = 0.907$) nor does vegetables consumption ($\rho = -0.045$, $p = 0.437$). More than half of adolescents consume two portions of fruits daily (rural vs urban: 53.40 %:53.20 %), which is low percentage. And less then half (rural vs urban: 32.90 %:40.50 %) consume two portions of vegetables daily, which is even lower percentage. Low consumption of fruits and vegetables affects food inadequacy, and daily habit of fruit consumption in another study made in Sarajevo (Hodžić&Smajić, 2012) showed that it is developed by 29.40 % of adolescents, which is referred to one portion of fruits (exp. an apple) two times a day while 28.70 % students consume fruits three times a day. Vegetables are consumed once a day by 37.80 % adolescents and 37.20 % twice a day.



Graph 2. Consumption of fruits and vegetables per portion among male adolescents according to the place of residence

Regarding male adolescents, fruit consumption per portion does not depend on the place of residence ($\rho = -0.044$, $p = 0.436$). Nor does vegetables consumption ($\rho = 0.077$, $p = 0.201$). Only half of participants (57.63 %) living in urban area and 49.15 % in rural area consume two portions of fruits, which does not present high percentage. At least two portions of vegetables a day consume only 31.67 % of rural adolescents and 41.22 % of urban. In relation to fruits, a higher percentage of adolescents declared that do not consume vegetables at all (urban vs rural: 14.12 %:16.67 %). In Croatia, 66.00 % of students do not eat fruit every day, and as much as 76.00 % do not eat vegetables every day (Ministarstvo

zdravlja R Hrvatske, 2013). In relation to the fruits, a lower percentage of adolescents in both areas consume vegetables regularly and occasionally, and it is recommended to consume one portion of vegetables more than the fruits.

Table 3. Daily consumption of vegetables/fruits

Sex	Place of Residence	Food	How many portions of vegetables/fruits do you eat per day?			Total
			Less	Recommended	More	
Female	Rural	Vegetables	N 44	22	3	69
			% 63.80	31.90	4.30	100.00
	Urban		N 148	42	21	211
			% 70.10	19.90	10.00	100.00
Male	Rural		N 38	11	7	56
			% 67.90	19.60	12.50	100.00
	Urban		N 165	47	37	249
			% 68.20	19.40	12.40	100.00
Female	Rural	Fruits	N 44	19	4	67
			% 65.70	28.40	6.00	100.00
	Urban		N 130	52	25	207
			% 62.80	25.10	12.10	100.00
Male	Rural		N 33	16	5	54
			% 61.11	29.63	9.26	100.00
	Urban		N 141	60	39	240
			% 58.75	25.00	16.25	100.00

The daily consumption of vegetables and place of residence of female adolescents are not correlated ($\rho = -0.037$, $p = 0.535$). Most of adolescent girls in both areas in the highest percentage consume vegetables less than recommended (rural vs. urban: 63.80 %:70.10 %). One-third of adolescent girls from rural (31.90 %) and one fifth from the urban areas (19.90 %) consume the recommended amount of vegetables. The daily consumption of vegetables expressed by number of portions does not depend on the place of residence of male adolescents ($\chi^2 = 0.002$, $p = 0.999$). The highest percentage of adolescents in both areas consume less than recommended a day (rural vs urban: 67.90 %:68.20 %). Less then 20.00 % consume the recommended amount of vegetables, 2-3 portions a day (rural vs urban: 19.60 %:19.40 %). Davis et al. (2008) point out that urban children consume a little more servings of fruits than rural ($p = 0.381$). Nevertheless, rural children eat a little more servings of vegetables than urban ($t(130) = -0.188$, $p = 0.851$). When analyzed together, portions of fruits and vegetables are higher in urban children than in rural ($t(130) = 0.424$, $p = 0.672$). The average number of daily servings was 2.14 (SD= 1.18, n= 97) for urban children and 2.07 (SD= 1.24, n= 35) for rural ($t(130) = 0.293$, $p = 0.770$). According to previous results (Table 1. and Graph 1.) a high percentage of adolescents (females and males) do not have a habit of consuming fruits every day. Daily consumption of fruits expressed by portions and place of residence of girls are not correlated ($\rho = 0.042$, $p = 0.490$) nor is considering boys ($\chi^2 = 1.846$, $p = 0.397$). Regarding daily frequency, it is certified that adolescents do not consume the recommended daily amount of fruits (one to two portions) which is consumed only by third of adolescent from rural areas (female vs. male: 28.40 %:29.63 %) and a quarter from the urban area (female vs. male: 25.10 %:25.00 %). Most adolescents (cca. 60.00 %) consume fruits in less than one portion a day. A small

percentage, but more pronounced in urban areas (female vs. male: 12.10 %:16.25 %) consume more than recommended (two portions of fruits per day).

Table 6. Weekly consumption of vegetables/fruits

Sex	Place of Residence	Food	How many times per week do you eat vegetables?				Total
			Less	Recommended	More	Never	
Female	Rural	Vegetables	N 17	21	10	2	50
			% 34.00	42.00	20.00	4.00	100.00
	Urban		N 54	52	43	3	152
			% 35.50	34.20	28.30	2.00	100.00
Male	Rural		N 19	19	56	6	100
			% 36.54	36.54	29.02	3.11	100.00
	Urban		N 63	68	12	2	145
			% 32.64	35.23	23.08	3.85	100.00
Female	Rural	Fruits	N 14	17	17	0	48
			% 29.20	35.40	35.40	0.00	100.00
	Urban		N 44	35	67	2	148
			% 29.70	23.60	45.30	1.40	100.00
Male	Rural		N 18	61	13	5	97
			% 34.62	30.65	25.00	2.51	100.00
	Urban		N 54	20	79	1	154
			% 27.14	38.46	39.70	1.92	100.00

In order to explore thoroughly the habit of eating fruits and vegetables, the survey included questions of consumed amount of fruits and vegetables on a daily and weekly basis. The weekly consumption of fruits and place of residence of girls are not correlated with one another ($\rho = 0.064$, $p = 0.372$). In the rural area, the largest and the same percentage of female adolescents consume fruits in recommended and more than recommended amount (35.40 %). Adolescent girls from urban areas most frequently consume fruits more than recommended (45.30 %). So, a third of rural and almost half of urban adolescents consume the recommended amount of fruits. The weekly consumption of fruits does not depend on the place of residence of male respondents ($\chi^2 = 0.126$, $p = 0.254$). Very low percentage of adolescents have a proper habit of eating fruits on a weekly basis (more than 10 times), and among them, there are more adolescents from urban areas (39.70 %). The weekly consumption of vegetables and place of residence of female adolescents are not correlated ($\rho = 0.018$, $p = 0.802$) nor is regarding male adolescent ($\rho = 0.043$, $p = 0.503$). Looking at the weekly consumption of vegetables, adolescents almost equally consume vegetables in given quantities and as expected, a little less than one-third consumes vegetables in the weekly recommended amount (23.08 % in the rural and 29.02 % in the urban area). Comprehensively looking, about 60.00 % of adolescents have sufficient consumption of vegetables per week, but the percentage should be higher. Adolescent girls from rural areas consume vegetables in the highest percentage as recommended (42.00 %). Urban adolescents consume it less than recommended in 35.50 %, although there is the approximate percentage of those who consume vegetables in recommended amount (34.20%). According to Dapi et al. (2005) vegetables/leafy vegetables are consumed in both areas, but are more consumed in urban areas. Bertić (2013) states that the fruits are consumed several times a week by 52.00 % of respondents, every day by 33.00 % and 15.00 % rarely. According to the survey, results shows that the consumption of fruit in

those participants is satisfactory and the same results are with the consumption of vegetables.

Conclusion

Statistical significance in relation to the sex and the place of residence was shown only for the habit of consuming fruits and vegetables among male adolescents, where is very high percentage of urban adolescents who do not have a mentioned habit (80.31%). Although there is no statistical significance, very high percentage of adolescents of both sexes and in both places of residence do not consume fruits nor vegetables regularly. Vegetables are recommended to be used in higher amount than fruits and these results show that are consumed even less than fruits. Consumption of fruits and vegetables was examined in different ways (by habits, portions daily and weekly) to make sure that the participants were thinking and calculating approximate amount of consumed food. Interventions of promoting fruits and vegetables are needed and schools are the most adequate place for that.

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STUDY OF TYPOLOGIES OF LIVER PATHOLOGIES IN SHEEP IN SLAUGHTERHOUSES

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Abstract

Liver is the largest gland in the body. It, is one of the most important organs because liver acts as a clearing house for substances that enter the body. Liver plays a major role in metabolism and has a number of functions, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, and hormone production. This study was carried out to determine the prevalence of liver lesions in sheep and to define the liver morphological alterations in sheep in slaughterhouses. From their macroscopic examination resulted that in 91 samples (40.6%) macroscopic changes have been verified, while 133 samples (59.4%) have not shown any macroscopic changes. In examined livers has resulted presence of different parasites. The prevalence rate of *Fasciola hepatica* and *Dicrocoelium dendriticum* was 28.60% (26 sheep samples) and 45.00% (41 sheep samples), respectively. *Echinococcus granulosus*'s cysts have been found in 17 samples (18.70%). Lesions more often seen are necrosis, fibrosis of liver capsule and around bile ducts, bile ducts enlargement etc,. Our study results showed that liver injuries in sheep slaughtered in Tirana abattoir is high and must be take measures in this field by improvement of management and environmental factors in fields.

Key words: Sheep, slaughterhouse, macroscopic examination, liver diseases.

Introduction

Albania has a livestock population of about 1.8 million sheep and livestock forms an important segment of the agricultural production. High prevalence of animal diseases affects both health and productivity of livestock population. Slaughterhouses provide an excellent opportunity for detecting diseases of both economic and public health importance (Raji et al., 2010). Diseases and lesions are grossly diagnosed based on pathological changes of organ color, size, morphology, consistency, presence of lesions and parasites (Mellau et al., 2010). The slaughterhouse and its regulations, represents a key control point of livestock production chain (Raji et al., 2010) and any observation and information obtained at slaughterhouse can contribute to the understanding of slaughtered animals' diseases. Routine examinations in slaughterhouses, in Albania, indicate presence of different pathologies in sheep livers. The main aims of the present study were to determine the frequencies of such condemnation, among the slaughtered sheep.

The importance and scope of the study

Liver plays a major role in metabolism and has a number of functions, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, etc (Stalker MJ and (Tony) Hayes MA, 2009).

A special feature of the liver, as a body, is the fact that its pathologies, in most cases, are not manifested clinically, and the damage that comes from their health and animal production, is great (Radostits O.M. et al. , 2009).

Study of liver pathology in sheep is important not only because they are frequent but also because some parasitic disease affecting humans, causing life-threatening injuries.

Many pathological processes of the liver appear coupled with each other. Recognition of this association, the appearance of their causal connections and a set of features, their coexistence in the concrete conditions sheep arrangement practice in our country, allows creating a full image right on the burden of liver pathological processes and, consequently, the adoption of a complex appropriate measures to prevent, treat and combat them.

Study in sheep liver pathological processes in Albania, related to different aspects; a number of processes have poor clinical expression; some of them related to pathogens and parasitic, viral or bacterial; others are based metabolic, etc.. Clarification of these aspects would be a concrete aid for veterinary practice in combating liver pathologies.

Materials and Methods

The study area and animals and sample collection: The study was carried out in different slaughterhouses in Tirana. A total of 224 sheep livers were controlled on the period June-November 2010. Animals were randomly chosen in slaughterhouses; the animals were of different age and origin. It was not possible to get the exact records on breed, gender and age for each slaughtered animals during the study period due to poor recording system at the abattoir. The liver and the other organs have been examined. Liver lesions were grossly diagnosed based on pathological changes of organ colour, size, morphology, consistence, presence of lesions and parasites. Pathologies with sporadic character such as cracks and liver displacement were not considered. For every liver examined macroscopically, a sample was taken and was put in formaline 10%. Microscopic samples were prepared in pathologic anatomy laboratory in Faculty of Veterinary Medicine, Thessaloniki and were stained with hematoxiline and eosine.

Data analysis: The data gathered software were analyzed with statistic software Splus 8 with the help of categorical data analysis capability of this software.

Results and Discussion

In this study have been sampled 224 sheep. In 91 samples (40.6%) macroscopic changes have been verified, while 133 samples (59.4%) have not shown any macroscopic changes.

In the end of liver macroscopic examination, in sheep have resulted different pathologic processes. All processes found in livers and their frequency are included on table 1.

Table 1. Pathologic processes found from macroscopic examination of liver in sheep

RANKING	TYPE OF PROCESS	NUMBER OF SAMPLES	
		No. of samples	%
1.	Dicroceliosis	41	45.00
2.	Fascioliasis	26	28.60
3.	<i>Echinococcus granulosus</i>	17	18.70
4.	Steatosis	1	1.10
5.	Microabscesses	2	2.20
6.	<i>Cysticercus tenuicollis</i>	4	4.40
TOTAL		91	100

From macroscopic examination of liver in 91 sheep with different ages, has resulted presence of some parasites such as *Echinococcus granulosus*, *Fasciola hepatica*, *Dicrocoelium dendriticum* dhe *Cisticercus tenuicollis*.

Pathologic processes more often seen are dicroceliosis and fascioliasis. High rates are reported in other countries too. (Rahim Ahmadi, Edrsi Mahdavi Sikejor, Morteza Maleki, (2010); Joseph C. Boray, March (2007); Mellau L.S.B.; Nonga H.E.; Karimuribo E. D. (2010); George V. Hillyer, Maricelis Soler de Galanes, Paola Buchón, Jostein Bjorland, (1996); (Regional Veterinary Laboratory Reports, Dublin (2009).

Presence of *Dicrocoelium dendriticum* and *Fasciola hepatica* parasites on some occasions is light. Areas with necrosis found in sheep are small sized of around 0.5 mm. The damage was caused is defined as hemorrhage in parenchyma and a low presence of *Dicrocoelium dendriticum* parasites on bile ducts. On bile ducts we do not perceive a change, while what it concerns to parenchyma, areas with red stripes are discerned that indicate parasites passage. The presence of fibrosis around bile ducts some times is minor while on some occasions is large. (Sissay et al., 2007; (Boray, (2007); Çela, (2007); Mohammad H. Movassagh Ghazani, et al., (2008). Also, an element which is often noticed and deep expressed on infestations from *Fasciola hepatica* is bile ducts enlargement. There are livers where exists only one enlarged bile duct, in some others are present a few of them. The size of bile ducts enlargement is various.

On incision surface *F. hepatica* parasites of different phases are noticed, some of them are unmaturred and some others matured.

Echinococcus cysts of different sizes have been present on 18.70% of occasions and concretely in 17 sheep samples. The presence on density and size of hydatide cysts is different. There have been found a few cysts joined together to create a bigger cyst. Hemorrhagic areas have been seen around cysts.

Steatosis was found in one sample. The liver is pale alike clay on colour with abnormal consistence. The liver is easily disjoined. Hemorrhagic areas are seen and on bile ducts there is presence of *D. dendriticum*.

Microabscesses are an other pathologic process verified during macroscopic examination. This process covers 2.2% of pathologic alterations occasions.

Cisticercus tenuicollis cysts are present in 4 samples.

These pathologic processes have not been found alone on all occasions. Different accompaniments of these processes have resulted on this study. Accompaniment of *D. dendriticum* and *F. hepatica*, but on other occasions, presence of *Echinococcus* cysts, *Cysticercus tenuicollis* cyst and a presence of *D. dendriticum* has been apparent.

Table 2. Accompaniments of different pathologies

TYPE OF PROCESS	<i>D. dendriticum</i>	<i>F. hepatica</i>	<i>E. granulosus</i>	Steatosis	Microabscesses	<i>C. tenuicollis</i>	TOTAL
<i>D. dendriticum</i>	15	14	8	1	-	3	41
<i>F. hepatica</i>	14	12	-	-	-	-	26
<i>E. granulosus</i>	8	-	8	-	-	1	17
Steatosis	1	-	-	-	-	-	1
Microabscesses	-	-	-	-	2	-	2
<i>C. tenuicollis</i>	3	-	1	-	-	-	4
TOTAL	41	26	17	1	2	4	91

Based on table 2 data where appear associations of different pathologies on real numbers, high level of *D. dendriticum* and *F. hepatica* parasites's association is present. It appears a variety of some associations of *D.dendriticum* with other pathologic processes. In 41 livers in which *D.dendriticum* parasites are present, only in 15 of them, they have been found alone. On other occasions they were associated with different pathologic processes. On 14 occasions where are present *D.dendriticum* parasites, there are present *F. hepatica* parasites too. Some times *D.dendriticum* parasites are associated with *Echinococcus granulosus* parasites, and concretely this happened on 8 occasions. In 3 livers, except the presence of *D.dendriticum* parasites was found a *Cysticercus tenuicollis* cyst as well. Association of *D.dendriticum* parasites with steatosis has appeared in only one occasion.

The presence of *F. hepatica* parasites in associations appeared only with *D.dendriticum* parasites. This association appears in 14 livers, where 26 livers are contaminated by fascioliasis. In 12 livers are present only *F. hepatica* parasites.

Smaller associations's figures appear on other processes. According to the table above appears that *Echinococcus granulosus* parasites are associated with *D. dendriticum* parasites on 8 occasions, while the association of *Echinococcus granulosus* parasites with a *Cysticercus tenuicollis* cyst appears in only one sample.

Steatosis is a process seen in only one liver where have been found *D. dendriticum* parasites as well.

Microabscesses which have been found in two samples, they are the only pathologic processes not associated with other processes. The presence of *Cysticercus tenuicollis* cysts appears in 3 samples associated with the presence of *D. dendriticum* and *Echinococcus granulosus* parasites.



Fig. 1. Liver infested by *D. dendriticum*

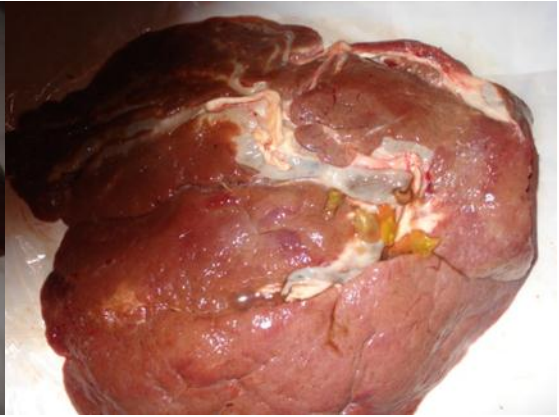


Fig. 2. Liver infested by *F. Hepatica*



Fig. 3. Liver infested by *E. granulosis*.



Fig. 4. Liver with steatosis and infested by Cyst with different sizes. *D. dendriticum*.

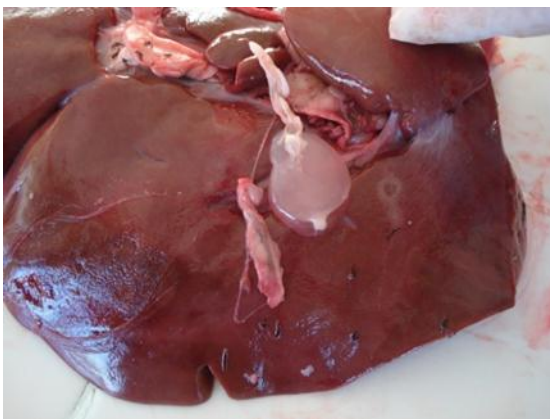


Fig. 5. Liver infested by *C. teniuicollis* and *D. dendriticum*.

Conclusions

This study indicated that the infestation scale of sheep liver in slaughterhouses is high. An important role on their appearance plays our country's climate conditions, a Mediterranean climate, which impulses the development of parasites biological cycle. Retrospective study of diseases encountered at abattoir will provide useful pathology profiles which could be useful in future risk assessment and planning of control and prevention strategies. It is therefore recommended that retraining of abattoir staff on modern methods of meat inspection practices be done. Proper record keeping of abattoir data and if possible computerization of such data is necessary.

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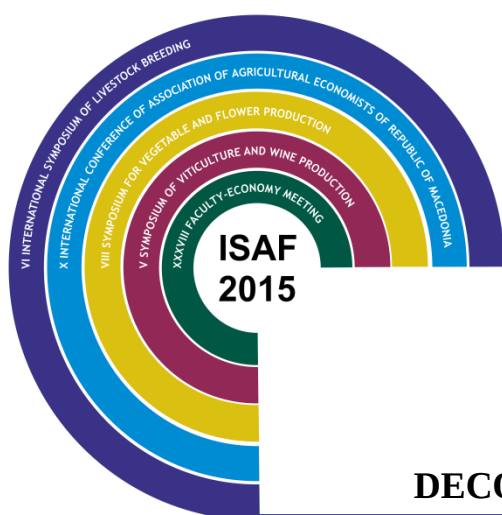
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SECTION 5. VEGETABLE, FLOWER AND DECORATIVE PLANTS PRODUCTION

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INCREASING THE LEVEL OF RESISTANCE TOWARDS BACTERIAL SPOT AND SPECK IN TOMATO CULTIVARS WITH HIGH BIOLOGICAL VALUE FRUITS

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Abstract

Developing of commercial cultivars with higher yield, biological value, taste and quality and improved resistance to biotic and abiotic stress factors is a major goal of tomato breeding programs. Breeding for disease resistant tomato varieties and their implementation in large-scale vegetable –growing is considered as the most efficient method of controlling foliar bacterioses in tomato crops. Pathogens' natural population of bacterial spot and speck on tomatoes (*Xanthomonas vesicatoria*, *X. gardneri* and *Pseudomonas syringae* pv. *tomato*) is heterogeneous in specie, pathotype and race. Interspecific hybrids IZK Alia and Plovdivska karotina were screened for foliage bacterial disease resistance and continuous individual-plant selection was conducted. As a result their resistance in the foliage to *X. gardneri* and *P. syringae* pv. *tomato* (R0,R1), *X. vesicatoria* (T1,T2,T3) was increased and become stable. These hybrids are resistant to multiple bacterial diseases in the foliage and they have fruits with high biological value.

Key words: varieties, resistance, *Xanthomonas vesicatoria*, *Pseudomonas syringae* pv. *tomato*, quality.

Introduction

Disease resistance is a major goal in breeding new varieties and plays a key role in vegetable crop production and integrated pest management practices. Identification of tomato genotypes and development of new varieties with genetic resistance to the most economically important diseases in the country is crucial for ensuring high, stable and ecologically clean production. Bacterial diseases reduce yields and fruit quality in tomato crops. Bacterial speck and spot caused by *Pseudomonas syringae* pv. *tomato*, *Xanthomonas vesicatoria* и *X. gardneri*, respectively are two of the diseases of economical importance for tomato crops in Bulgaria (Bogatzevska, 2002; Kizheva *et al.*, 2013). Pathogens' natural populations of *Xanthomonas vesicatoria*, *X. gardneri* and *Pseudomonas syringae* pv. *tomato* are heterogeneous in specie, pathotype and race. Race 0, 1 of *P. syringae* pv. *tomato* and race T1, T2, T3 of *X. vesicatoria* are wide spread in tomato fields throughout the country (Bogatzevska, 2002; Bogatzevska *et al.*, 1989, 2000; Bogatzevska and Sotirova, 2002; Kizheva *et al.*, 2011; Stoyanova *et al.*, 2014, 2015).

Obtaining healthy output of tomatoes is difficult because of the biology of pathogens: they can remain and be transmitted with tomato and weed seeds; they can grow symptomless in the tomato foliage and they are housing in the weeds (Jones *et al.*, 1986; Mariano and McCarter, 1991; Bogatzevska, 2002; Bogatzevska and Nikolova, 2003; Agrawal *et al.*, 2012, Sharma and Agrawal, 2014). Bacterial diseases incident, develop and distribute with cyclic peaks in tomato crops (Bogatzevska, 2002).

Using of resistant varieties in tomato growing is a highly efficient, economic and ecological approach in pest management. An intensive shift change in commercial tomato varieties requires screening to bacterial pathogens of the newly released ones. Bulgarian tomato varieties (hybrids and cultivars) used for commercial vegetable-growing in the country are not resistant to *X. gardneri*, *P. syringae* pv. *tomato* and *X. vesicatoria*. Variety “Plovdivska karotina” possess multiple disease resistance to race of *P. syringae* pv. *tomato* and race T1 на *X. vesicatoria*. Var. “IZK Alia” of cherry type is resistant to race R1 and medium sensitive to race R0 of *P. syringae* pv. *tomato* (Aleksandrova *et al.*, 2013). There are no commercial varieties with resistance to the causal agents of bacterial spot and speck worldwide so far (Scott *et al.*, 1997; 2001, 2003).

This research aimed to study the possibilities of obtaining durable resistance to *X. gardneri*, *P. syringae* pv. *tomato* (R0, R1) and *X. vesicatoria* (T1, T2, T3) trough continuous individual plant selection in var. “Plovdivska karotina” and in var. “IZK Alia” having their variety authenticity sustained.

Materials and methods

Plant material – var. “Plovdivska karotina” (Ganeva and Pevicharova, 2011a) and var. “IZK Alia” (Ganeva and Pevicharova, 2011b), releases of Vegetable Crops Research Institute “Maritsa”- Plovdiv.

Bacterial pathogen - *P. syringae* pv. *tomato*: strain № 31pt of race R0; strain № 27pt of race R1; *X. vesicatoria*: T1-strain № 24t; T2- strain № 53t and T3-strain № 30t; *X. gardneri* strain № 67t (strains belong to pathology collection of the Plant protection department of ISSAPP “N. Pushkarov”, Sofia).

Inoculation in vivo – plants from each variety (over/or 20 numbers) in growth stage of the 5-6th leaf were infected with bacterial suspension of *P. syringae* pv. *tomato* from 24h culture in concentration 10⁴ cfu/ml) and *X. vesicatoria* from 36h culture in concentration 10⁸ cfu/ml) by vacuum-infiltration method. The infected plants were grown individually in plots with nutrient solution of Knop at room temperature (Bogatsevska, 2002). Continuous selection of individual plants resistant to of *P. syringae* pv. *tomato* (Pst), races R0 and R1, *X. vesicatoria* (Xv) races T1, T2, T3 and *X. gardnerii* (Xg) was made and then inoculations were performed following the pathogens’ order: strait - R0/R0, R1/R1 and crossed - R0/R1; R1/R0; XvT1/T2; XvT3/T2; XvT2/ Xg; Xg/XvT2.

Evaluation of the disease – the development of hypersensitive reaction (HR) was registered after 24h; the number of the spots necessary to diagnose bacterial spot and speck diseases on the leaves was registered 4-5 days after the infiltration. The mean score of infestation was calculated (ms) based on the scale of Chambers and Merriman (1975) for *P. syringae* pv. *tomato* and the scale of Sotirova and Beleva (1975) for *X. vesicatoria* and *X. gardneri*. The classification of the varieties in groups was made on the basis of the mean score of infestation (ms): immune - 0 (I); resistant: 0,01-0,6(R); medium sensitive: 0,61-1,49(MS); sensitive: 1,50-2,99(S); highly sensitive over 3(SS). Healthy plants and with HR were

selected and transmitted to be grown on the experimental field at Maritsa VCRI. Seeds were obtained for further screening. Agro-economical, morphological and biochemical assessments were performed under three-year comparative field trails. Standard seeds produced at VCRI “Maritsa”, Plovdiv were used for controls.

The results obtained were processed statistically by analysis of variance (Lakin, 1990) and multiple analyses of variance (Duncan, 1955).

Results and discussion

Variety “Plovdivska karotina” and var. “IZK Alia” were released through interspecific hybridization. Wild species *Solanum chilense* and *Solanum racemigerum* were involved in the pedigree of the both varieites, respectively. Var. “Plovdivska karotina” has indeterminate growth habit, semi-large fruits (70-80 g) with intensive orange color and round shape. Featuring a unique combination and high content of three components with antioxidant effect - vitamin C, lycopene and β -carotene, they are therefore particularly suitable for healthy nutrition and production of functional foods. Variety “IZK Alia” belongs to the “cherry” type tomatoes; the fruits are with oval-elongated shape and intensive red colour; firm and tasty with enriched content of total sugars, acids, vitamin C and lycopene. Obtaining high yields and output with good morphological, biochemical and sensory characteristics of the fruits is a prerequisite in developing disease resistant varieties. Sources of disease resistance are wild species, lines and varieties developed through interspecific hybridization (Scott *et al.*, 1989). The objects of resent study are var. “Plovdivska karotina” and var. “IZK Alia” which involved in their pedigree accessions from wild species with previously determined resistance to bacterial spot and speck (Sotirova and Bogatzzevska, 1994).

Screening to *P. syringae* pv. *tomato*, race R0 of the plants from var. “Plovdivska karotina” resulted in 40,9% of healthy and HR-plants and 59,1% of sensitive plants with score of 1 (table 1). The average spot number per plant was five. Screening to race R1 of *P. syringae* pv. *tomato* showed that 76,7% of the tested plants from the same variety were healthy and with hypersensitive reaction, and 23,3% of them were sensitive with score of 1 (average spot number per plant – 0,2 five). Selection of individual plant resistant to both races of *P. syringae* pv. *tomato* was performed twice and the number of healthy and HR-plants increases with 55,1% and 15,3% for race R0 and R1, respectively. The number of plants with score 1 was reduced for both races. Due to the individual selection of resistant plants mean score (ms) was considerably reduced in both races, compare to the initial plant materials from var. “Plovdivska karotina”. The resistance to bacterial spot and speck diseases was increased and stabilized. Two individual plant progenies were selected - lines LP.k PstR0 and LP.k PstR1.

In var. “IZK Alia” screening to *P. syringae* pv. *tomato*, race R0 resulted in 28,6% of healthy and HR-plants and 71,4% of sensitive plants with score of 1 (table 1). Records from the screening to race R1 of *P. syringae* pv. *tomato* were 33,3% and 66,7% for the healthy and HR-plants and sensitive plants, respectively. As a result of the individual selection percentage of healthy and HR-plants increased with 53,2% (up to 81,8%) for race R0 and with 19,3% (up to 52,6%) for race R1. Percentage of sensitive plants with score 1 and number of spots per plant were lower for race R0 compared to race R1. Mean scores of infection to race R0 and race R1 were lower than 0,6 in the selected lines from var. “IZK Alia” which defined them as resistant ones.

Table 1. Resistance of tomato varieties to *P. syringae* pv. *tomato*

Seed origin	PstR0								PstR1							
	Number of plants	0	HR	1	2	3	4	ms	Number of plants	0	HR	1	2	3	4	ms
Pl. karotina	22	4	5	13	0	0	0	0,59	30	2	21	7	0	0	0	0,23
R0, R1	26	13	5	8	0	0	0	0,31	28	12	6	10	0	0	0	0,36
R0, R1	25	5	19	1	0	0	0	0,04	25	13	10	2	0	0	0	0,08
R0									26	13	0	9	4	0	0	0,15
R1	25	3	3	19	0	0	0	0,76								
R0,R1,R0R1, R1R0	22	0	20	2	0	0	0	0,09	26	5	11	10	0	0	0	0,38
IZK Alia	21	0	6	6	7	2	0	1,24	21	2	5	14	0	0	0	0,67
R0, R1	20	4	0	15	1	0	0	0,85	20	2	6	12	0	0	0	0,60
R0, R1	33	16	11	6	0	0	0	0,18	19	6	4	9	0	0	0	0,53
R0									29	9	15	5	0	0	0	0,17
R1	19	3	13	3	0	0	0	0,16								
R0,R1,R0R1, R1R0	37	31	1	5	0	0	0	0,14	35	26	4	5	0	0	0	0,14

R0, R1- seeds derived from healthy and HR – plants to race R0 or R1

R0, R1, R0R1, R1R0 – a mixture of seeds collected from individually selected plants

In order to develop line with complex resistance to both races of *P. syringae* pv. *tomato*, experimental work proceeded with the selected individual resistant plants to races R0 and R1 (healthy and with HR-reaction) from both varieties (tabl.1). Line LP.k PstR0 and LP.k PstR1 from var. “Plovdivska karotina” were cross-infected with race R1 and R0, respectively and although an increase of the mean scores was recorded they still remained considerably low. Plants with score of 3 and 4 were not recorded. Multiple resistance to both races of *P. syringae* pv. *tomato* was increased and stabilized in line LP.k Pst selected from var. “Plovdivska karotina” as a result of the individual race screening and cross-testing to both races.

Lines with resistance to race R0 or R1 selected from var. “IZK Alia” were cross-infected with race R1 and R0, respectively and their mean scores remained considerably low (table 1). Multiple resistance to both races of *P. syringae* pv. *tomato* was increased and stabilized in line линия LAlya Pst. Healthy and HR-plants were recorded in both varieties after artificial inoculation with three races of *X. vesicatoria* (table 2.) First screening with standard seeds form var. “Plovdivska karotina” showed resistant reaction to race T1 and medium sensitive reaction to race T2 and T3.

Unlike variety “IZK Alia” individually healthy plants from var. “Plovdivska karotina” were selected at the first testing to the three races. The resistance to race T1 sustained after the second artificial inoculation. A hundred per cent resistance was achieved to race T3. The most virulent race in the experiment was T2 towards which no resistant lines have been developed worldwide so far. Some resistance to race T1 and T3 was found in line Hawaii 7998 and Hawaii 7981, respectively (Scott et al., 2003). Resistance to race T2 was increased after cross-tests following individual plant selection. Selected line LP.k XvT1T2T3 from var. “Plovdivska karotina” was resistant to races T1 and T3 and medium sensitive to race T2. Further selection for resistance to race T2 is necessary in this line to become more durable.

Variety “IZK Alia” was resistance to race T3, sensitive to race T2 and medium sensitive to race T1 at the first inoculation of standard seeds. No plants with healthy and HR-reaction

to races T1 or T2 were recorded after the first test. Selection of individual plant with healthy and HR reaction to race T3 was made. Their progenies were used for further screenings to race T1 and T2 and continuous selection for resistant individual plants. In the cross-tests there were recorded an increase in the medium scores of infection for race T1 and decrease for races T2 and T3, having durable resistant reaction. Line LAlya Xv was selected having a resistant reaction to the three races of *X. vesicatoria*. Resistance to *X. vesicatoria* is not always related to a hypersensitive response (HR). The resistance to races T1 and T3 of *X. vesicatoria* is based on a HR that is controlled by avirulence genes *avrRxv* and *avrXv3* in the pathogen and corresponding avirulence genes in the host *Rxv* and *Xv3*. Genotype PI114490 was reported to be resistant to race T2 without HR. currently there are no commercial tomato varieties with resistance to bacterial spot worldwide (Scott *et al.*, 2003). In our study the reaction of resistance to race T2 was found to be with HR and healthy plants, having healthy plants prevailed. The resistance to *X. gardneri* in var. “Plovdivska karotina” becomes stable due to continuous individual selection after artificial inoculation. Selection for bacterial spot disease had biggest achievement after inoculation of individually selected healthy plants to race T2 of *X. vesicatoria*. Line LAlya Xg from var. “IZK Alia” was selected as resistant to *X. gardneri*. The number of plants with score of 1 is insignificantly low with single spots and mean score of infection under 0,6 (table 3).

Table 2. Resistance of tomato varieties to *X. vesicatoria*

Seed origin	T1								T2								T3							
	N	0	HR	1	2	3	4	ms	N	0	HR	1	2	3	4	ms	N	0	HR	1	2	3	4	ms
Pl. karot.	21	9	9	3	0	0	0	0,14	20	3	5	10	2	0	0	0,70	22	0	7	10	5	0	0	0,91
T1,T2,T3	22	17	2	3	0	0	0	0,14	22	8	0	13	1	0	0	0,68	20	18	2	0	0	0	0	0,00
T1									25	10	1	14	0	0	0	0,56								
T3									20	16	3	1	0	0	0	0,05								
Xg									32	12	0	17	3	0	0	0,72								
Xv,Xg	24	16	4	4	0	0	0	0,17	20	0	7	7	6	0	0	0,95	20	8	10	2	0	0	0	0,10
IZK Alia	21	0	0	15	6	0	0	1,29	21	0	0	4	16	1	0	1,86	21	5	3	13	0	0	0	0,62
T1, T2,T3	18	14	3	1	0	0	0	0,06	30	14	3	13	0	0	0	0,43	27	7	14	6	0	0	0	0,22
T1									19	5	4	10	0	0	0	0,53								
Xv,Xg	20	6	5	9	0	0	0	0,45	28	20	6	2	0	0	0	0,07	21	9	8	4	0	0	0	0,19

N- Number of plants

T1/ T2/T3- seeds derived from healthy and HR –plants to race T1, T2 or T3, respectively

Xv, Xg-a mixture of seeds collected from individually selected healthy and HR- plants to Xv T1,T2,T3 and Xg

Table 3. Resistance of tomato varieties to *X. gardneri*

Seed origin	var. “Plovdivska karotina”								var. “IZK Alia”							
	Number of plants	0	HR	1	2	3	4	ms	Number of plants	0	HR	1	2	3	4	ms
Standard seeds	21	5	0	11	5	0	0	1,00	20	9	0	11	0	0	0	0,55
Xg	27	17	6	4	0	0	0	0,15	35	9	7	19	0	0	0	0,54
T2	20	20	0	0	0	0	0	0,00								
Xv T1T2T3, Xg	20	2	1	17	0	0	0	0,85	22	14	4	4	0	0	0	0,18

Xg- seeds derived from healthy and HR –plants to Xg

Xv, Xg-a mixture of seeds collected from individually selected healthy and HR-plants to Xv T1, T2, T3 and Xg

Three lines from var. “Plovdivska karotina” and three lines var. “IZK Alia” with resistance to *P. syringae* pv. *tomato*, *X. vesicatoria* and *X. gardneri* were selected and grown in comparative field trails for agro-morphological assessment for three seasons. No

significant differences were observed in the duration of the vegetation period (table 4). No differences in early yield, total yield and morphological characteristics of the fruits were recorded between the selected resistant lines and the corresponding variety of origin (table 4 and 5).

Variation (CV) is within the limits. There have been no significant changes in agro-economic and morphological characteristics among the lines selected from var. "Plovdivska karotina" and var. "IZK Alia" despite of the continuous selection for resistance to bacterial spot and speck. They remained unchanged in terms of the basic chemical components (table 6). Their high biological value, based on the antioxidants as lycopene, β -caroten and ascorbic acid stayed stable.

Table 4. Agro-economic assessment of tomato varieties and lines

Variety/ line	Days to maturity		Early yield, kg/da		Total yield, kg/da	
	$\bar{X} \pm sd$	CV%	$\bar{X} \pm sd$	CV%	$\bar{X} \pm sd$	CV%
var. "Pl. karotina"	107 $\pm$ 1,61ns	1,52	1406,7 $\pm$ 63,25ns	4,50	4493,5 $\pm$ 317,98ns	7,08
LP,k Pst	107 $\pm$ 1,43ns	1,33	1421,7 $\pm$ 59,10ns	4,16	4392,5 $\pm$ 282,60ns	6,43
LP,k Xv	108 $\pm$ 1,52ns	1,41	1392,0 $\pm$ 66,30ns	4,76	4522,5 $\pm$ 276,34ns	6,11
LP,k Xg	107 $\pm$ 1,40ns	1,31	1417,0 $\pm$ 59,26ns	4,18	4615,5 $\pm$ 208,71ns	4,52
var. "IZK Alia"	100 $\pm$ 1,45ns	1,45	1226,0 $\pm$ 115,01ns	9,38	3504,0 $\pm$ 205,89ns	5,88
LAlya Pst	99 $\pm$ 1,56ns	1,59	1311,0 $\pm$ 70,47ns	5,38	3591,0 $\pm$ 219,21ns	6,10
LAlya Xv	98 $\pm$ 1,29ns	1,32	1260,0 $\pm$ 121,19ns	9,62	3690,0 $\pm$ 149,78ns	4,05
LAlya Xg	99 $\pm$ 1,51ns	1,50	1303,1 $\pm$ 58,14ns	4,46	3550,0 $\pm$ 239,63ns	6,75

a,b.. Duncan`s multiple range test ($p < 0,05$)

Table 5. Fruits morphological and physical characteristics of tomato varieties and lines

Variety/ line	Average fruit weight (g)		Fruit shape I=h/d		Pericarp thickness (cm)		Length of pedicle hole,(cm)		Pressure resistance (kg)	
	$\bar{X} \pm sd$	CV%	$\bar{X} \pm sd$	CV%	$\bar{X} \pm sd$	CV%	$\bar{X} \pm sd$	CV%	$\bar{X} \pm sd$	CV%
Pl.karotina	82,7 $\pm$ 5,54ns	6,70	0,95 $\pm$ 0,01ns	1,30	0,56 $\pm$ 0,52ns	9,22	0,69 $\pm$ 0,14ns	19,86	6,08 $\pm$ 0,77ns	12,71
L P,k Pst	80,0 $\pm$ 5,98ns	7,48	0,95 $\pm$ 0,01ns	1,88	0,57 $\pm$ 0,48ns	8,47	0,64 $\pm$ 0,97ns	15,09	6,34 $\pm$ 0,96ns	15,15
L P,k Xv	82,1 $\pm$ 4,89ns	5,95	0,96 $\pm$ 0,02ns	2,19	0,59 $\pm$ 0,74ns	12,51	0,72 $\pm$ 0,11ns	15,77	6,08 $\pm$ 0,82ns	13,53
L P,k Xg	78,9 $\pm$ 5,84ns	7,40	0,94 $\pm$ 0,01ns	1,22	0,63 $\pm$ 0,67ns	10,71	0,76 $\pm$ 0,09ns	12,71	7,06 $\pm$ 0,72ns	10,15
I ZK Alia	15,6 $\pm$ 0,89ns	5,73	1,45 $\pm$ 0,04ns	2,65	0,46 $\pm$ 0,05ns	11,90	0,58 $\pm$ 0,13ns	22,48	5,30 $\pm$ 0,50ns	9,43
L Alya Pst	15,4 $\pm$ 1,14ns	7,40	1,43 $\pm$ 0,05ns	3,27	0,50 $\pm$ 0,07ns	14,14	0,62 $\pm$ 0,11ns	17,65	5,70 $\pm$ 0,65ns	11,57
L Alya Xv	15,4 $\pm$ 0,89ns	5,80	1,44 $\pm$ 0,04ns	2,95	0,48 $\pm$ 0,04ns	9,32	0,66 $\pm$ 0,09ns	13,55	5,24 $\pm$ 0,85ns	16,28
L Alya Xg	15,2 $\pm$ 1,30ns	8,58	1,45 $\pm$ 0,05ns	3,71	0,44 $\pm$ 0,05ns	12,45	0,58 $\pm$ 0,08ns	14,43	5,74 $\pm$ 1,02ns	17,88

a,b.. Duncan`s multiple range test ($p < 0,05$)

Table 6. Chemical components of tomato varieties and lines

Variety/line	Brix (%)	Titrateable organic acids (%)	Ascorbic acid (mg %)	Total pigments (mg %)	Lycopene (mg %)	β-caroten (mg %)
Pl. karotina	5,7	0,30	51,80	6,80	2,80	3,40
LP.k Pst	5,4	0,35	43,84	7,97	3,62	3,86
LP.k Xv	5,3	0,27	56,53	9,36	3,96	4,75
LP.k Xg	5,4	0,30	40,88	9,80	4,98	3,93
$\bar{X} \pm sd$	5,45±0,17	0,31±0,03	48,14±7,29	8,48±1,36	3,84±0,90	3,99±0,56
CV%	3,18	10,87	15,15	16,11	23,50	14,1
IZK Alia	9,5	0,80	50,2	10,0	6,5	
LAlia Pst	8,8	0,63	52,1	8,7	7,0	
LAlia Xv	9,0	0,75	53,2	10,0	7,0	
LAlia Xg	9,2	0,80	54,5	10,2	7,2	
$\bar{X} \pm sd$	9,10±0,30	0,75±0,08	52,50±1,82	9,72±0,71	6,93±0,30	
CV%	3,27	10,77	3,47	7,30	4,31	

a,b.. Duncan's multiple range test ($p < 0.05$)

Conclusions

Resistance to *P. syringae* pv. *tomato* (R0, R1), *X. vesicatoria* (T1, T2, T3) and *X. gardneri* was increased and stabilized having continuous individual selection applied in var. "Plovdivska karotina" and var. "IZK Alia". The resistance is based on healthy and HR-plants. Lines LP.k Pst, LP.k Xv and LP.k Xg from var. "Plovdivska karotina" and lines LAlia Pst, LAlia Xv и LAlia Xg from var. "IZK Alia" were selected with multiple resistance and unchanged agro-economical, morphological and biochemical characteristics. Selected lines are new sources of resistance to bacterial spot and speck in tomatoes, having high agro-economic value.

Acknowledgment

The results presented in the paper are an output from research project B02/4-2014 „Ecological methods and measures for control of viral and bacterial diseases of vegetable crops from *Solanaceae* family for quality production“, funded from National Science Fund under Bulgarian Ministry of Education and Science.

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UDC: 635.64(497.712)
Original scientific paper

THE EFFECT OF COLORED SHADE NETS ON TOMATO YIELD PRODUCED IN SKOPJE REGION

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Abstract

The effect of colored shade nets on tomato yield grown in open field in Skopje region in 2014 was the main aim of this investigation. Two tomato hybrids for summer production (Optima F1 and Izmir F1) were used in the field experiment. The follow treatments in the experiment were evaluated: I. red shade net with 40 % of shading intensity, II. white shade net with 40 % of shading intensity, III. green shade net with 40 % of shading intensity and IV. without shade net (control). Colored shade nets were installed after 35 days of transplanting. Height of plants, diameter of fruits, pericarp size and total yields were measured during the vegetation. According to the obtained results of tomato yield, there were significant differences between the treatments with color shade nets and control treatment. Namely, the treatment with red shade net showed the highest yield of 77.8 t/ha, then comes the treatment with white shade net (69.7 t/ha) and then the treatment with green shade net (55,7t/ha), while the lowest yield (43.1 t/ha) was achieved in control treatment.

Keywords: Colored shade nets, tomato, yield

Introduction

Tomato (*Lycopersicon esculentum* Mill) is one of the main vegetable crops in the R. Macedonia with 5746 hectares and production of 160 530 tones per year (State Statistical Office 2015). The practice of using shade nets in vegetable production in the country is pretty actual in the last few years. Shade nets in vegetable production are usually used during the summer, when temperatures and direct sun radiation are at the highest level, so its obtain protection of plants thus the physiological strain of plants and fruit.

Effects of climate changes are another important reason to find and use new tools and technologies in vegetables production. Global warming effects that are already setting in are likely to increase the incidence of tomato abiotic disorders as the climate changes. Causes of physiological disorders include genetic susceptibility, environmental factors, irrigation practices, nutrition, and cultural practices such as pruning and training (Milenković et al., 2012). The use photo selective nets as a new technology in the production from the beginning are understood as managing of direct light parts of the spectrum that basically enhance characteristic of the culture. Potential of the new technology of photo-selective shade netting improve the productivity, quality and harvest

time of horticultural crops (Shahak, 2008). Colored shading nets are improving or control: vegetative growth, branching, flowering, fruit maturation, the color of the fruits chemical composition of the fruits and water demands of the plants. There are differences in fruit quality were observed in tomatoes grown in plastic houses compared to those grown in open field conditions or between fruits from screen-house (colored shade nets) and plastic-house integrated with color shade nets (Ilić et al., 2012).

To choose the proper colored net it is important to know the reaction of the plants to the different wavelengths of the solar spectrum, in physiological processes and in the process of photosynthesis. Ultra violet part (200-320nm) is not useful for plants and has no influence to the physiological processes but causes burns and whitening of the leaves. Blue part (400 – 500nm) is necessary for control and growing, allows absorption of carotenes and chlorophyll and strongly influences process of photosynthesis. Red part of the spectrum (600-700nm) is important for the growth and development of plants, lacking in this section often appear deficiencies in plants. Physiologically there is reduced synthesis of chlorophyll witch strongly influences process of photosynthesis. Intensity of the red part of the spectrum (700-750nm) is very important because it improves blooming, strengthens and lengthens the stem cells. Blocking of this part of the solar spectrum significantly slows plant growth. Part of the spectrum with wavelengths above 750 nm, the light energy is converted into heat energy.

Materials and methods

The main aim of this research was to evaluate tomato yield grown in open field and under the different color nets. The experiment was established in Skopje region (Latitude 42.07689, Longitude 21.44329) in 2014. The Optima F1 and Izmir F1 tomato hybrids were used in the field experiment. The follow treatments were evaluated: I. red shade net with 40 % of shading intensity, II. white shade net with 40 % of shading intensity, III. green shade net with 40 % of shading intensity and IV. without shade net (control). The nets were installed on 2.5 meter high construction with aim to provide adequate quantum of light. The experiment was designed according to randomized block design for experimental purposes in 3 replications. The size of each plot (replication) was 13 m² (35 plants in one replication or 0.75 m spacing between the rows and 0.5 m plant spacing in the row).

The transplanting date was May the 15th. Colored shade nets were installed after 35 days of transplanting (June 20th). Soil analyses were realized before establishing of field experiment. It was noticed that large quantity of macro elements and microelements were required for nutrition. The irrigation of the experiment was scheduled according long-term average daily evapotranspiration of tomato in Skopje region by FAO software CROPWAT, using crop coefficient (K_c) and stage length adjusted for local condition. Irrigation of all treatments was carried out with drip irrigation system, while fertilizers were applied through the system. Also, common pest were observed and treated adequately. Height of plants, diameter of fruits, pericarp size and total yields were measured during the vegetation.

The data for yield were subjected to statistical analysis of variance and means were compared using the least significant difference (LSD) at the 5% level of probability ($P < 0.05$) test.

Results and Discussion

The tomato crop needs a lot of heat during the whole growing period. If temperature is below 15°C the flowering stops and if temperature drops below 10°C the growth stops. At temperatures higher than 25°C life processes in tomatoes decrease, and at temperatures above 30°C they completely stop. During the vegetation, the minimum temperatures range from 13.6 to 16.4°C, while maximum temperatures from 23.4 to 29.2°C (Table 1).

Table 1. Temperatures during the vegetation period (°C)

Months	Minimum	Maximum
V (May 15 days)	10 -13	20 -23
VI (June 30 days)	12 -15	22 -29
VII (July 31 days)	15 -18	25 -34
VIII (August 31 days)	16 -18	26 -33
IX (September 13 days)	15 -18	24 -27
Average temperatures (°C)	13,6 -16,4	23,4 -29,2

Data presented in the Table 2 shows that the year 2014 was very humid with a lot of rainfalls during the growing season (455 mm) which is rather unusual for the Skopje region. 51 days of the vegetation period was characterized by frequent precipitation, while 69 days without precipitation.

Table 2. Precipitation during vegetation period (mm)

Months	mm	days	
		precipitation	without precipitation
V (May 15 days)			
VI (June 30 days)	40	7	8
VII (July 31 days)	65	12	18
VIII (August 31 days)	125	17	14
IX (September 13 days)	115	7	24
Months	110	8	5
Total	455	51	69

Table 3. Height of plants, diameter of fruits and pericarp size

Treatments	Height of plants (cm)	Diameter (cm)		Pericarp (mm)
		height	width	
Control (open field)	104.1	6.1	6.4	4.1
Treatment I (red nets)	124.6	6.9	7.2	4.6
Treatment II (white nets)	124.5	6.6	7.1	5.0
Treatment III (green nets)	122.7	6.7	7.2	4.4

The treatments I, II, and III shows highest plants in comparison with control treatment IV. Similar results were obtained with the diameter of the tomato fruits. Regarding the size of tomato pericarp, the largest pericarp was achieved in treatment II.

Table 4. Yield per harvest (kg/ha) and differences in % between control and treatments

Harvest	Control (Open field)	Treatment I (Red net)	Treatment II (White net)	Treatment III (Green net)	Vegetative period (in days)
1	1365,89	1725,93	1550,67	1432,56	73
2	10355,60	17545,09	15910,59	12584,46	81
3	11628,14	18002,29	16649,73	12192,02	89
4	9201,17	15601,98	15640,08	11662,43	98
5	6061,72	15640,08	13296,93	11239,52	106
6	4492,00	9296,42	6705,61	6610,36	120
Total	43104,52 ^d	77811,79 ^a	69753,62 ^b	55721,36 ^c	
%	100	180	160	130	

*Values in rows followed by the same letter are not significantly different at the 0.05 probability level

Even that all vegetation period was followed by temperature changes and rather unusual and intensive rainfall, the first harvest has started only after 73 days from transplanting. The highest yields in the first three harvests were achieved in treatments I, II and III which can be connected with the technology of the shading. Different cultivation practice were investigated for shortening the period from transplanting to the first's harvests in some vegetables in our country, as: inert media combined with drip fertigation in tomato crop (Petrevska, 1999), drip fertigation of tomato crop (Tanaskovic, 2005), drip fertigation and different pepper pruning technology (Tanaskovik et al., 2015).

In the present investigation, the treatment with red shade net showed the highest total yield of 77.8 t/ha, then comes the treatment with white shade net (69.7 t/ha) and then the treatment with green shade net (55.7 t/ha), while the lowest yield (43.1 t/ha) was achieved in control treatment. All three treatments with shade nets show statistically significant differences at 0.05 level of probability when compared to the control treatment. Also, there was a statistically significant difference among the treatments with shade nets. When we compared shade nets treatments with control treatment in relatively values, it can be concluded that treatment I resulted in 80% higher yield, treatment II in 60% and treatment III in 30 %. Tanaskovik, Cukaliev and Dimov (2015) presented higher yields in red shade net compared with white shade net pruned pepper grown in plastic house. Also, same authors reported better yields in tomato crop grown in plastic house and under different color nets compared with tomato crop without net and grown in plastic house.

If we compared the average yield of all treatments with the yield of each treatment separately, than we can concluded that only first and second treatments shows yields over the average (Figure 1).

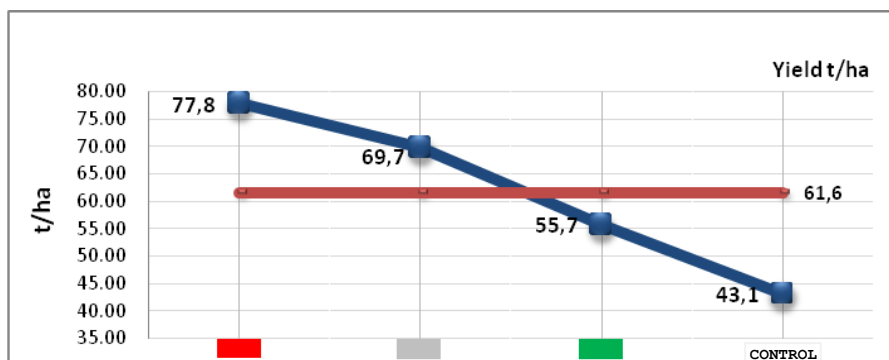


Figure 1. Comparison of average yield of all treatments with the yield of each treatment separately

Conclusions

The results of this research showed significant results of tomato production under color shade nets (red and white) compared to production without shade net (control treatment). Therefore, this technology could be recommended in the production of tomatoes in open field production in Republic of Macedonia, especially in the future period, when possible negative effects of climate changes are expected to have influence on vegetable production.

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UDC: 635.64_293.8
Original scientific paper

SENSORY CHARACTERISTICS AND BIOLOGICAL VALUE OF TOMATO LINES WITH RESISTANCE TO BACTERIAL SPECK AND SPOT

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Abstract

Eight tomato breeding lines with resistance to bacterial speck and spot were evaluated for their biological value and sensory profile aiming to find appropriate parental components for future breeding. Sufficient combinations between soluble solids and antioxidants (ascorbic acid and lycopene) were obtained. Moderate correlations were found between sourness and content of titratable organic acids and between sweetness and soluble solids. From breeding point of view the investigated tomato lines possessed high biological value but their flavour was not good enough. However some of them could be used as parental components in combinative breeding for high quality. Comparatively lower evaluations were given to the texture due to the mushiness. The future selection with these lines has to be focused predominantly on the texture - to increase the firmness to the level of becoming acceptable.

Key words: *Solanum lycopersicum* L., *Pseudomonas syringae* pv. *tomato*, *Xanthomonas vesicatoria*, antioxidants, flavor.

Introduction

Tomatoes are one of the important vegetable crops providing healthy components for human body. They are a natural source of three vital antioxidants – lycopene, beta-carotene and ascorbic acid. The regular consumption of tomatoes and tomato products correlate to a reduction in risk of contracting various types of cancer and cardiovascular diseases (Giovannucci, 1999; Borguinia and Da Silva, 2009).

Nowadays the efforts of tomato breeders are directed to combine consumer's desires with producer's ones. Developing new hybrids with high biological value and improved sensory profile meets the consumer's requirements. Increasing the levels of natural antioxidants by selecting tomato genotypes with antioxidant nutritional qualities is one of the main goals of the contemporary breeding programmes (Frusciante et al., 2007; Kuti and Konuru, 2005; Grozeva et al., 2014).

To be marketable, the new varieties and hybrids have to possess good taste which is the combination of the interaction between sugars, acids and a set of approximately 30 volatile compounds (Tieman et al., 2006). Some literatures suggest that breeding tomatoes for high

levels of sugars and acids will result in varieties with improved flavour (Jones and Scott, 1984; Stevens et al., 1979; Pevicharova and Todorov, 2001). Despite the efforts of tomato breeders, fresh tomatoes often do not meet the high standards of flavour required by consumers (Speirs et al., 1998).

In order to meet producer's requirements, increased yield and resistance to economically important diseases are on the focus. Commercial Bulgarian tomato varieties are not resistant to bacterial speck and spot (Aleksandrova, et al., 2014). These diseases reduce yields and compromises the nutritional and organoleptic qualities of the crop output (Bogatzevska and Sotirova, 2002; Gitaitis, et al., 1992). Developing new resistant hybrids is a promising way for obtaining healthy and high quality tomato production.

The objective of this study was to evaluate newly released tomato lines resistant to bacterial speck and spot on their sensory profile and biological value aiming to find appropriate parental components for future breeding work.

Materials and methods

Plant material

The experiment was carried out at the Maritsa Vegetable Crops Research Institute (VCRI), Plovdiv during the period 2013-2015 under field conditions. Eight tomato breeding lines were investigated in the experiment. Seven lines were developed at the Institute of Soil Science, Agro-Technology and Plant Protection "Nikola Pushkarov", Sofia in collaboration with the Institute of Plant Physiology and Genetics, Sofia. Lines L358/1, L358/2, L358/3, L44, L404 possess multiple resistance to race R0 and R1 *Pseudomonas syringae* pv. *tomato* and races T1, T2 and T3 of *Xanthomonas vesicatoria*; line L380 to races T1 and T3; line L384 to races T2, T3 of *Xanthomonas vesicatoria*. Line H7998 (resistant to race T1) and line H7981 (resistant to race T3) had been used for the race differentiation for *Xanthomonas vesicatoria*.

Seeds of the tomato genotypes were sown and pricked in heated green-house. The seedlings were transplanted under open field conditions at the beginning of May. The experiment was carried out with 10 plants of each genotype. Yield per plant and average fruit weight were calculated.

Analytical evaluation

Analysis of the chemical composition and sensory evaluation of the fruits were performed at the Laboratory for Vegetable Quality Control of Maritsa VCRI. Samples of 10 mature fruits were analysed for following components: soluble solids using a digital refractometer; titratable acid content calculated as citric acid after titration with 0.1 N NaOH; ascorbic acid by Tilman's reaction with 2, 6-dichlorophenolindophenol (Genadiev et al. 1969); total pigments and lycopene by Manueyan (1991).

Sensory evaluation

The sensory analysis was performed on the following traits: appearance, shape, external colour, internal colour, aroma, peel tenderness, visible cellulose fibres, sourness, sweetness, texture, and overall taste. The panel test was carried out by five constant panelists using a five-point hedonic scale from 1 to 5 with 0.25 step.

Data analysis

The results were processed by Duncan's multiple range test (1955) and correlation analysis (SPSS software). The coefficient of variability (CV) and the standard deviation (sd) were also calculated (Lakin, 1990).

Results and Discussion

The average fruit weight of the investigated tomato lines varied in large limits. Lines L358/3 and L404 were of cherry type (Table 1). The biggest size of the tomato fruits was measured in L380. All the other lines formed considerably smaller fruits compared to L380. The yield per plant ranged significantly such as the average fruit weigh. According to the coefficients of variability the lines were of good uniformity in both characters.

The predominant part of the studied tomato lines were distinguished by their high values of the investigated basic chemical components (Table 2). They were close in total pigments and lycopene but more different in soluble solids and ascorbic acid. All the lines were of lycopene type with over 74 percent of lycopene content of the total pigments.

Line L380 had the lowest values of the studied ingredients. Sufficient combinations between soluble solids and antioxidants (ascorbic acid and lycopene) were obtained in the other tomato lines. The high contents of antioxidants classify them as tomato lines with high biological value. Lines L404, L358/3 and H7998 were the most valuable ones.

Table 1. Yield per plant and average fruit weight

Breeding line	Yield per plant (kg)	CV (%)	Average fruit weight (g)	CV (%)
	$\bar{x} \pm sd$		$\bar{x} \pm sd$	
L358/1	1076.5 $\pm$ 95.9 ef	8.9	20.85 $\pm$ 2.96 e	14.2
L358/2	1972.5 $\pm$ 130.2 b	6.6	12.85 $\pm$ 1.95 f	15.21
L358/3	1047.5 $\pm$ 60.8 ef	5.8	7.95 $\pm$ 0.76 g	9.55
L44	1457.5 $\pm$ 139.4 cd	9.6	65.30 $\pm$ 5.79 b	8.86
L404	896.3 $\pm$ 112.5 f	12.6	8.33 $\pm$ 0.61 g	7.34
L380	3237.5 $\pm$ 231.7 a	7.2	165.15 $\pm$ 12.20 a	7.39
L384	1107.5 $\pm$ 125.0 e	11.3	53.05 $\pm$ 4.85 c	9.14
H7998	1542.5 $\pm$ 113.8 c	7.4	31.80 $\pm$ 4.07 d	12.80
H7981	1343.8 $\pm$ 71.3 d	5.3	32.90 $\pm$ 2.75 d	8.36

a. b. c... - Duncan.s multiple range test ($p < 0.05$)

Table 2. Basic chemical components in the studied tomato lines

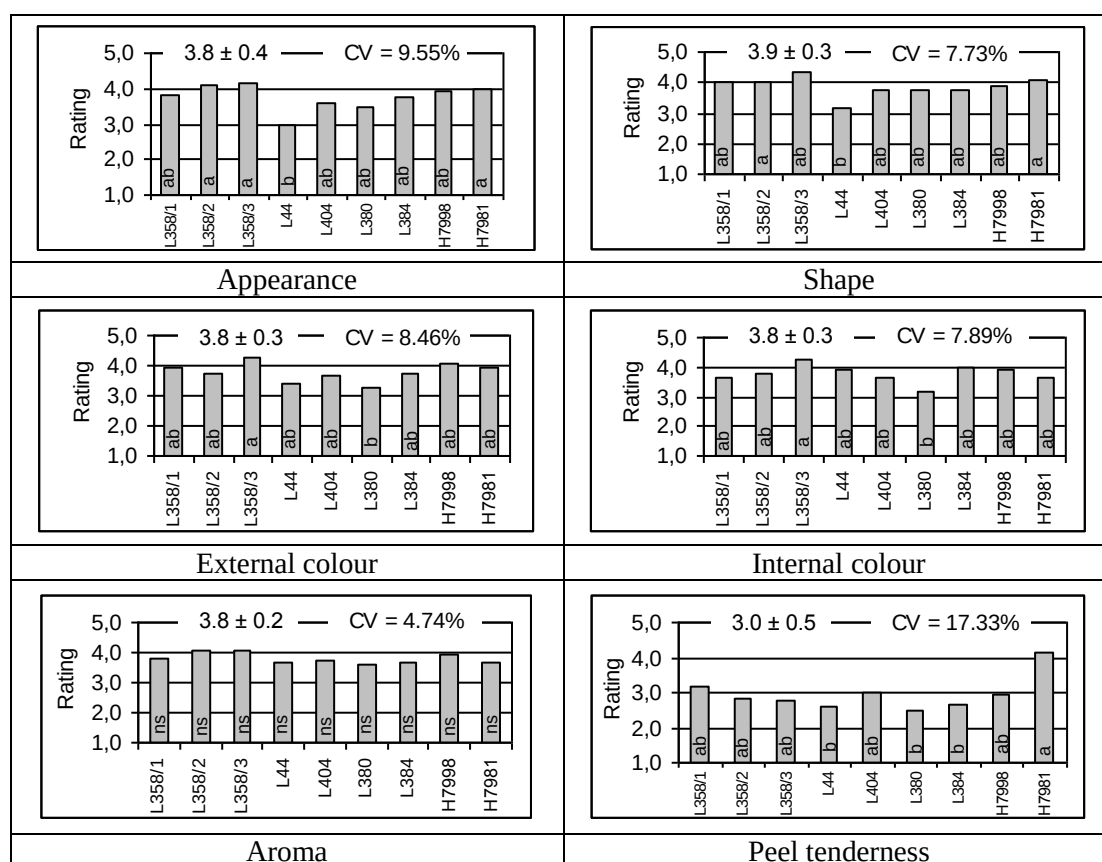
Breeding line	Soluble solids (%)	Ascorbic acid (mg per 100 g)	Titrateable organic acids (%)	Total pigments (mg per 100 g)	Lycopene (mg per 100 g)
L358/1	6.7 ^b	47.83 ^b	0.46 ^{bcd}	8.00 ^a	6.96 ^a
L358/2	6.8 ^c	48.20 ^b	0.58 ^a	7.95 ^a	7.07 ^a
L358/3	8.5 ^a	67.13 ^a	0.40 ^{de}	7.98 ^a	7.06 ^a
L44	5.6 ^c	33.67 ^{cd}	0.44 ^{cd}	7.65 ^a	6.86 ^{ab}
L404	9.1 ^a	70.01 ^a	0.53 ^{ab}	7.91 ^a	6.83 ^{ab}
L380	4.3 ^d	29.20 ^d	0.34 ^e	6.32 ^b	4.67 ^b
L384	4.8 ^d	41.57 ^{bc}	0.35 ^e	6.79 ^b	6.10 ^{ab}
H7998	5.7 ^c	63.37 ^a	0.51 ^{de}	8.07 ^a	7.22 ^a
H7981	6.1 ^{bc}	43.83 ^{bc}	0.57 ^a	6.57 ^b	5.66 ^{ab}
$\bar{x} \pm sd$	6.4 $\pm$ 1.59	49.42 $\pm$ 14.51	0.46 $\pm$ 0.09	7.47 $\pm$ 0.70	6.49 $\pm$ 0.85
CV (%)	24.94	29.36	19.57	9.37	13.10

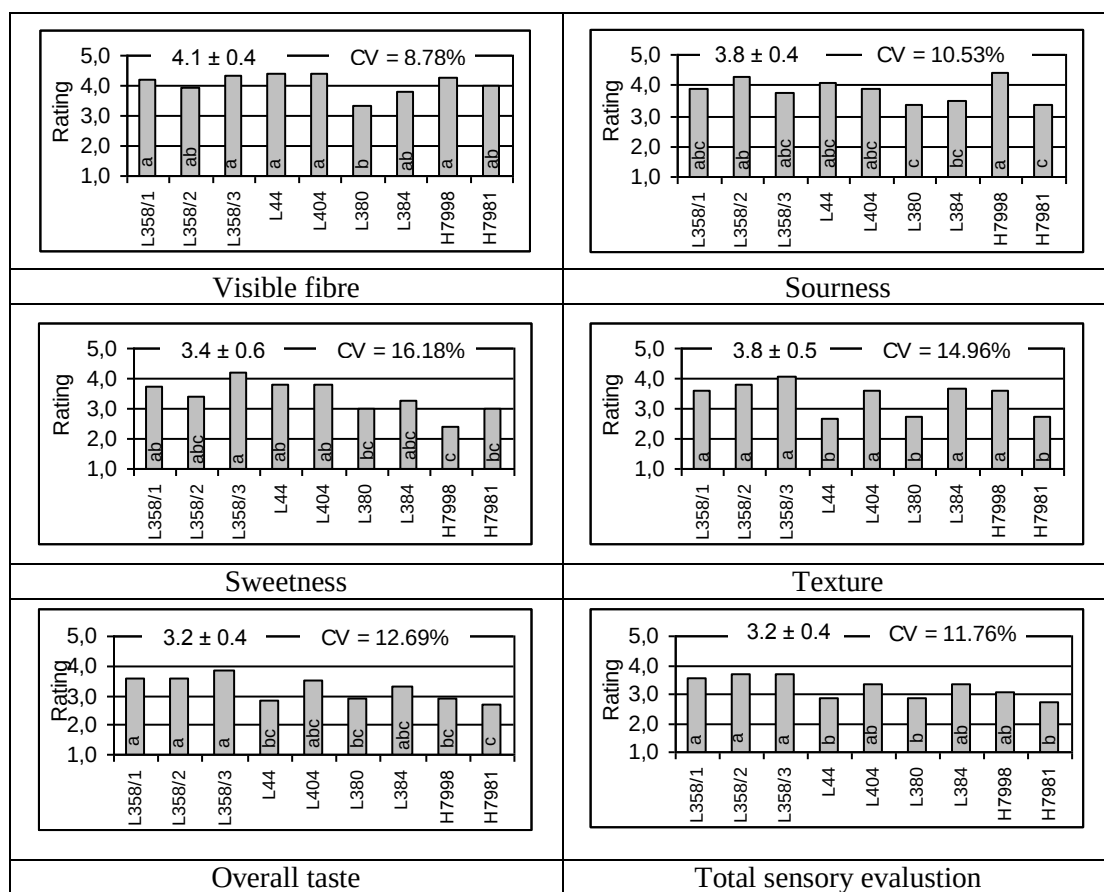
a. b. c... - Duncan.s multiple range test ($p < 0.05$)

Aroma was the most stable trait reported on the panel test in the studied tomato lines (Figure 1). All the lines had not too saturated but typical aroma. Coefficient of variability less than 10 percent was established for appearance, shape, colour and size of the visible cellulose fibres. Fruits of the each line were healthy, uniform in shape and size except of L44. Concerning external and internal colour statistical differences were proved only between L358/3 and L380. The highest sensory assessment was given to the visible fibre. More cellulose fibres in the tomato flesh were observed only in L380.

The lines had more different expression in respect to their gustatory sensory characteristics. As a whole, the perception for sourness exceeded the perception of sweetness. Comparatively lower evaluations were given to the texture due to the mushiness. It was of friable nature, atypical and not acceptable.

The low values of the basic chemical components reflected on the overall taste and on the total sensory evaluation for L380. The panelists defined its sensory profile as not good enough because of the wild aroma, not intensive internal colour, rough peel, soft texture, weak perception of sourness and sweetness.





a. b. c... - Duncan's multiple range test ($p < 0.05$). n.s. – not significant

Figure 1. Sensory analysis of the studied tomato lines

The total sensory evaluations for lines L44 and H7998 were close to L380. The poor very soft texture even in the unripe tomato fruits was the reason for their low assessments.

The total sensory evaluation was in moderate correlation with soluble solids, content of the total pigments and lycopene, external colour, visible fibres and sweetness (Table 3). It was in very strong correlation with texture and overall taste. Moderate correlations were found between sourness and content of titratable organic acids and between sweetness and soluble solids. Analogous results were reported by another scientists (Stevens et al., 1979; Pevicharova, 2002) for a variety of tomato types. The established correlations will permit us to replace one character with another within correlative couple in order to accelerate the process of the assessment for developing of tomato varieties with resistance to bacterial speck and spot.

Table 3. Correlations between some biochemical and sensory parameters

	Soluble solids	Titrateable acids	Total pigments	Lycopene	External colour	Internal colour	Visible fibres	Sourness	Sweetness	Texture	Overall taste	Total sensory evaluation
Soluble solids	♦											
Titrateable acids	0.428	♦										
Total pigments	0.652*	0.393	♦									
Lycopene	0.589*	0.422	0.954**	♦								
External colour	0.489	0.255	0.488	0.561*	♦							
Internal colour	0.418	0.024	0.529*	0.699*	0.717**	♦						
Visible fibres	0.771**	0.640*	0.856**	0.890**	0.744**	0.645*	♦					
Sourness	0.297	0.502*	0.873**	0.858**	0.258	0.377	0.679*	♦				
Sweetness	0.624*	-0.160	0.372	0.343	0.072	0.397	0.297	0.015	♦			
Texture	0.559*	0.045	0.616*	0.620*	0.732**	0.615*	0.698*	0.400	0.306	♦		
Overall taste	0.669*	-0.018	0.568*	0.504*	0.466	0.420	0.580*	0.248	0.661*	0.862**	♦	
Total evaluation	0.586*	0.116	0.640*	0.614*	0.599*	0.499	0.692*	0.391	0.502*	0.914**	0.955**	♦

Conclusions

The studied tomato lines possessed high biological value but their flavour was not satisfactory for breeding objectives. However some of them could be used as parental components in combinative breeding for high quality. Future selection with these lines has to be focused predominantly on the texture - to increase the firmness to the level of becoming acceptable. Obviously, it is quite difficult and complicated to combine the resistance to bacterial speck and spot with high content of antioxidants and good flavour. Nevertheless, quality control and tomato breeding in this direction must go in parallel.

Acknowledgment

The results presented in the paper are an output from research project B02/4-2014 „Ecological methods and measures for control of viral and bacterial diseases of vegetable crops from *Solanaceae* family for quality production“, funded from National Science Fund under Bulgarian Ministry of Education and Science.

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UDC: 635.64-181.198
Original scientific paper**EFFICIENCY OF BIOSTIMULANTS APPLICATION IN THE
REGULATION OF TOMATO PRODUCTIVITY**Todorović V.^{1*}, Zeljković S.¹, Koleška I.¹, Bosančić B.¹, Klokić I.²¹Faculty of Agriculture, University of Banja Luka, Republika Srpska, BiH²Syngenta Agro d.o.o., 78400 Gradiška, Republika Srpska, BiH

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Abstract

Agricultural bio stimulants include diverse formulations of compounds, substances and other products that are applied to plants or soils to regulate and enhance the crop's physiological processes, thus making them more efficient. Type of biostimulans and time of application is of particular importance. The aim of this study was to investigate the effect of the application of bio-stimulant Viva and Megafol on the productivity parameters (fruit weight (g) and total yield (kg m⁻²)) of two hybrid tomato (cv. Bostina F1 and Ombeline F1). Biostimulans were applied in two levels of nutrition: variant 1- standard nutrition, commonly used in farmers production and variant 2 - standard nutrition reduced about 40%. The highest increase in fruit weight was obtained under application of bio-stimulant Megafol with reduced nutrition, where the average fruit weight was 138.79 g. The lowest fruit weight was obtained under the standard nutrition with no application of biostimulant showing an average weight of tomato fruit about 121.22 g. Also, it was found that the lowest yield was under the standard nutrition with no application of biostimulant (5.73 kg m⁻²), and the highest yield was obtained under reduced nutrition with the application of Megafol biostimulant (7.13 kg m⁻²). Based on these results, it can be concluded that the application of biostimulant in tomato production is justified only in reduced nutrition application variant.

Keywords: tomato, fruit weight, yield, Viva, Megafol.**Introduction**

Tomato (*Lycopersicon esculentum*, Mill.) is one of the most important vegetables species in the world, not only for its huge consumption rate, but also for its high nutritive value provided by its antioxidants (lycopene, carotenoids, beta carotene etc.) with its positive influence on human organism (Preedy and Watson, 2008).

Tomato production in protected area of tunnel type is one of the leading ways of production in Republic of Srpska, Bosnia and Herzegovina. For this purpose are used mostly the indeterminate type hybrids which provide higher yield and better quality fruit. However, greenhouses being technically unequipped, the plants are under abiotic and biotic stress (Kaur and Gupta, 2005) which brings to certain physiological disorders and with it to yield decrees. One of the ways to decrease stress consequence is application of biostimulans together with other agro-technical measures in the course of production which Borowsky (2010) established in his research on cucumbers where plants which were

treated with biostimulans showed more stress resistance to low temperature stress. Beside that, in the last several years there is a trend to decrease mineral addition in plant nutrition, before all the nitrogen, phosphorus and potassium (Haytova, 2013) where the biostimulans play a significant role.

Biostimulans are comprised of physiological active matter helping plants in growth and development. By synergetic activity its components influence the system soil-plant-root (Paradjikovic et al., 2008). Biostimulans comprised of humic acids, amino-acids, proteins, peptides, polysaccharides and vitamins complex actively assist in root development and by that increase the resistance of root to the stress conditions. Foliar biostimulans based on amino-acids (prolin and triptophan) increase photosynthetic activity in plants aiding the quick overcoming of decreased plant growth caused by inadequate environmental conditions (Berlyn and Sivaramakrishon, 1996).

Many researcher asserted huge importance of positive biostimulans influence to increase yield and product quality on large amount of horticultural species including tomato (Abetz, 1989.; Gajc-Wolska et al., 2010). Research of Vinkovic et al., (2009) have indicated positive influence of application of biostimulants to root mass increase as well as leaf mass in tomato after transplantation with larger increase in variety stimulating the root development. Djuric et al., (2014) have also established that application of biostimulans leads to statistically significant increase in fresh root mass, plant mass and leaf mass, as well as increase in leaf surface, chlorophyll content and carotene content.

The goal of this research was to study the influence of application of two different biostimulans on the production parameters (fruit mass and total yield) of two tomato hybrid grown in the protected area of tunnel type.

Materials and methods

The research is conducted during the 2013 (april-august) on a family property in Sije, Tesanj municipality in Federation of Bosnia and Herzegovina. The experiment was set in a typical protected area of tunnel type covering 200 square meters with two tomato hybrids (Bostina F1 and Ombelina F1, Syngenta) with application of two biostimulans (Viva and Megafol, VelagroS.p.A., Italy) containing polysaccharides, glycosides and proteins, and are enriched with amino-acids, vitamins and microelements in chelate form. As such those biostimulants affect plant growth and development resulting in yield increase. The plantings were produced without application of biostimulans. The transplantation was conducted on 01.04.2013 in pots with 3 liters volume. Pot soil is acquired by mixing a commercial soil (Klassman Deilman, Germany) and garden soil in ratio 1:1. The analysis has indicated that the soil was with high content of organic matter, with pH 6.3 which is considered optimum for growth and development of tomato (Djurovka et al., 2006; Paradjikovic, 2009).

The plant nutrition was conducted through the system of fertirrigation with soluble nutrients (Yara, Finland) and based on recommendations related to soil content. Biostimulans Viva was applied in the soil through irrigation system in concentration 0,25% in the beginning of flowering and then every 30 days, while Megafol was applied in foliar manner by spraying in concentration of 0,20% every 15 days during the vegetation.

The experiment was set up as split-plot with three repetitions, with 8 plants for each treatment combination, where influence of the type of biostimulans was studied (Viva and Megafol) and type of nutrition (100% of recommended nutrition based on soil examination

taken as a control and 40% of the recommended nutrition) with two hybrids (Bostina F1 and Ombeline F1). The following combinations were studied in the course of the research:

- 100% of the recommended fertilization
- 100% of the recommended fertilization + Viva
- 100% of the recommended fertilization + Megafol
- 40% of the recommended fertilization
- 40% of the recommended fertilization + Viva
- 40% of the recommended fertilization + Megafol

Productivity related characteristics (fruit mass and total yield) were followed up to the fifth level. The data were analyzed by general linear modeling in statistical software package SPSS 22 (IBM 2013). Post-hoc analysis in cases of statistically significant differences ($p < 0.05$) were carried out by using Tukey's test ($p < 0.05$).

Results and discussion

Productivity related characteristics (fruit mass and total yield) were measured and analyzed.

Fruit mass

Fruit mass is a characteristic that to high extent defines the yield. This is the characteristic that makes producers decide between the available hybrids or agro-technical measure. Fruit mass is highly heritable characteristic related to a variety of hybrid, but also one of characteristics that can be influenced by applied technology of production (Bile *et al.*, 2008). Parray *et al.*, (2007) established that influence of any applied technology of production of the fruit mass is established, as the proper mineral nutrition increases the fruit mass in most varieties and hybrids.

The influence of application of biostimulators to the average fruit mass of the studied tomato hybrids is displayed in the Table 1 and Graph 1.

Average fruit mass of Bostina hybrid was 135.09 grams, and Ombeline hybrid 122.94 grams, which represents statistically highly significant difference in average fruit mass ($F=16.39$, $p < 0.001$). Different treatments of additional plant stimulators also gave different effects on average fruit mass, with indicative statistical difference ($F=2.97$, $p=0.07$). There were no other statistically significant differences or significant interactions.

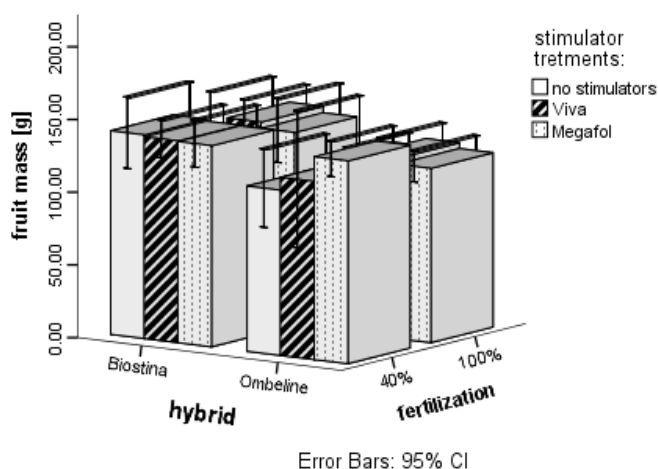
Table 1. Average fruit mass and variation [g] of tomato in relation to applied treatments in hybrids

	Bostina F1		Ombeline F1		Mean	F
	100%	40%	100%	40%		
nostim.	123.79± 6.93	139.95± 5.68	118.66 ± 1.91	113.53 ± 6.23	123.98	2.97 ⁱ
Viva	137.17± 3.22	138.64± 2.99	123.76 ± 2.92	122.45 ± 9.94	130.51	
Megafol	132.83± 5.09	138.20± 3.72	119.84 ±2.48	139.39 ± 2.38	132.57	
Mean	135.09		122.94			
F	16.39**					

It is notable that among analyzed combinations of treatments the smallest fruit mass was recorded with 100% recommended fertilization and no additional stimulators in Ombeline

F1 hybrid (118.66 grams) and largest fruit with the 40% of recommended fertilization and without additional stimulators in Bostina F1 hybrid (139.95 grams).

The results are in accordance with the results of Trofin and Onescu (2009) and Horgos et al., (2012) who also established the same principal in relation to the fruit mass regarding the application of biostimulators.



Graph 1. Influence of the applied biostimulans on the fruit mass

Yield

More recent trend in providing results regarding the yield is to measure the yield per plant or per m^2 as it represents the total fruit mass. Every hybrid is characterized by different yield and the differences in yield at the same hybrid by application of various production technologies are best to provide through yield in the unit of surface.

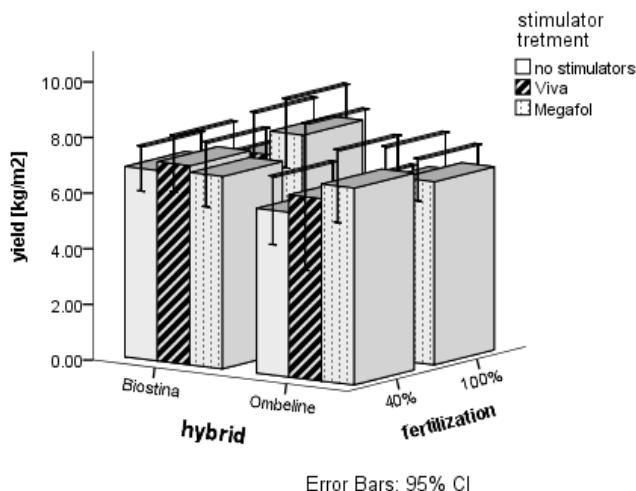
Table 2. Average yield and variation [kg/m²] of tomato in relation to applied treatments in hybrids

	Bostina F1		Ombeline F1		Mean	F
	100%	40%	100%	40%		
nostim.	5.98 ± 0.15	6.84 ± 0.18	5.47 ± 0.19	5.91 ± 0.28	6.05 ^a	11.41 ^{**}
Viva	6.82 ± 0.34	7.15 ± 0.23	6.30 ± 0.31	6.55 ± 0.61	6.70 ^b	
Megafol	7.67 ± 0.28	6.91 ± 0.26	6.58 ± 0.17	7.07 ± 0.31	7.06 ^b	
Mean	6.89		6.31			
F	11.10 ^{**}					

* Letter marks are groupings according to Tuckey's test

From the Table 2 and Graph 2 it is visible that average yield of Bostina was 6.89 kg/m² and Ombeline 6.31 kg/m² which is statistically highly significant difference in average yield (F=11.10, p=0.003). Different treatments with biostimulants resulted in statistically highly significant difference in yield (F=11.41, p<0.000). Following significant F test, In accordance with post-hoc Tukey's test analyzed stimulator treatments can be divided into two distinct groups, one without stimulator treatments and the second one with applied bio stimulator treatment. In the second group i.e. where Viva and Megafol biostimulants were respectively applied there was no statistically significant difference between those two

types of stimulators. There were no other statistically significant differences or significant interactions.



Graph 2. Influence of applied biostimulators on the yield

The positive influence of biostimulants to the plant productivity, i.e. the yield as the final results of tomato growing and production are described by many authors in their research (Dobromilska and Gubarewicz, 2008; Trofin and Oniscu, 2009; Gajc-Wolska et al., 2010), Kossak and Dyki (2008) in two-year research on biostimulants in tomato production established that biostimulants have significant contribution on yield and quality of tomato, but it depends on the applied biostimulants. This study has also confirmed such conclusions in terms that application of biostimulants resulted in higher yield. However there was no difference between the applied stimulators. There was also no difference between the full 100% application of recommended soil nutrition and 40% reduction in both fruit mass and the yield.

Conclusions

Biostimulants treatments in this research have significantly and positively influenced the yield of studied tomato hybrids in given conditions. In all treatment combinations better results were achieved with application of biostimulants.

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THE INFLUENCE OF FERTILIZER TYPE ON THE SEEDLINGS QUALITY OF PELARGONIUM HORTORUM L. H. BAIL.

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Abstract

The main aim of this research was to determine the influence of different types and solution concentrations of crystal fertilizers on the seedlings quality of *Pelargonium x hortorum* L. H. Bail., hybrid "Ringo 2000 deep scarlet". Easily soluble fertilizers were used in the following composition: NPK 9-10-34+M.E.; NPK 14-7-28+5CaO+M.E. and NPK 14-10-26+3MgO+M.E. According to the methodology of work and the purpose of the research, to determine the seedlings quality, the following biometric parameters were examined from 2010 to 2012: length of the stem, length of the root, thicknesses of the stem, mass of the plant, number of brunches and number of inflorescences. From the results of all examined biometrical parameters, it can be concluded that the nutrition with the crystal fertilizer NPK 14-10-26+3MgO+M.E., with dosage of 1,6 g/l solution and with dose increased to 3,2 g/l in full growth roots phase, has shown the best results. Plants fertilized with NPK 14-10-26+3MgO+M.E. have the highest length of the stem for about 59 % in full growth phase. The highest length of the root for about 54% in comparison with control treatment, have the plants fertilized with NPK 9-10-34+M.E. The highest thickness of the stem for about 26% in comparison with control treatment show plants fertilized with NPK 9-10-34+M.E. The highest mass of the plant for about 102% in comparison with control treatment was achieved in the plants fertilized with NPK 14-10-26+3MgO+M.E. The highest number of branches (141%) and number of inflorescences (25%) compared with control treatments show plants fertilized with NPK 14-10-26+3MgO+M.E.

Key words: *Pelargonium x hortorum* L. H. Bail., seedling, fertilizer, biometric parameters.

Introduction

Pelargonium genus has about 400 species and most of them come from South Africa (Шилиянова, 2005). Species of the genus *Pelargonium* are perennial plants, but they are often grown as annual plants. They feature a long blooming during the whole summer, until the appearance of autumn frost. The optimal pH for the substrate of "zonal" geraniums should range from 5.6 to 6.0 (Hammer, 1991 and Bethke, 1993). This is of particular significance because of the availability of the elements and the needs of the plants for certain elements. Geraniums have a great need for magnesium and calcium. Therefore, the regime of nutrition is based on a combination of KNO₃ and Ca (NO₃)₂ (Dole and Wilkins, 1999).

Also, studies indicate that the temperature and shape of the elements used in nutrition, affect the quality of seedlings. Thus, Ganmore-Neumann and Hagiladi (1992) found that the quality of the cuttings of *Pelargonium zonale* "Empress" and "Rubin" depends on the temperature and form of nitrogen added to the parent plants. At low temperatures, it is convenient to provide a higher concentration of NH_4 , while at higher temperatures it is convenient to provide a higher concentration of NO_3 . The best quality seedlings of *Pelargonium x hortorum*, series Maverick (red) are obtained by, at a dosage of 3 g/l using easily soluble fertilizer Scotts (Osmocote Exact) (Vujošević et al., 2009).

Therefore, the main purpose of this research is to examine the influence of different fertilizer types on the quality of seedlings of floral species *Pelargonium x hortorum* L. H. Bail. hybrid "Ringo 2000 deep scarlet".

Materials and Methods

The research was done in "Flower-Garden" farm in the village Vladevci, Strumica, Republic of Macedonia from 2010 to 2012. *Pelargonium x hortorum* L. H. Bail., hybrid "Ringo 2000 deep scarlet", a commercial substrate that is primarily used to produce seedlings of flowers and vegetables and easily soluble crystalline fertilizers with composition of: NPK 9-10-34+M.E.; NPK 14-7-28+5CaO+M.E. and NPK 14-10-26+3MgO+M.E. were used as examination material.

The seedlings of *Pelargonium x hortorum* L. H. Bail., were obtained from the Dutch company Syngenta. They were produced from seeds and seedlings with formed cotyledons leaves. Transplanting was made immediately after the purchase of seedlings, in traditional manner. Each plant was individually removed from the cell and transplanted in pots with a 9,5 cm diameter.

The flowerpots were previously filled with a substrate with trade name Tref.. Immediately after the transplanting, one irrigation application rate with 155 ml water was realized in each pot. The nutrition of the seedlings of *Pelargonium x hortorum* L. H. Bail., was initiated when the first two to three true leaves appeared. The nutrition was performed once a week, while the control treatment was only irrigated with 80 ml water (without nutrition). The treatments used in this research are presented in Table 1.

By the first two feedings, the treatments were fertilized with dosage of 1.6 g / l solution. The dosage of fertilizer in all treatments was doubled (3.2 g / l solution) after the second fertilization, i.e. in the phase of full rooted seedlings. The double dosage was based on the increased vegetative mass and consequently the increased need for nutrients. With double dosage the treatments were fed six times.

Table 1. Treatments used in the research

Treatments	Fertilizer
I	Without fertilizer Ø
II	Crystal NPK 9-10-34+M.E.
III	Crystal NPK 14-7-28+5CaO+M.E.
IV	Crystal NPK 14-10-26+3MgO+M.E.

Seedlings were produced at optimum conditions necessary for production and standard measures of care were applied: irrigation, ventilation, protection from diseases and pests. After 70 days from the transplantation in pots, by random selection, 30 plants of each

treatment were measured. According to the purpose of the research, the following biometric parameters were examined: length of the stem, length of the root, thickness of the stem, mass of the plant, number of branches and number of inflorescences.

The results were statistically processed with the method of analysis of variance and LSD test.

Results and Discussion

The highest average value for the length of the stem (113 mm) was obtained in plants fertilized with crystalline fertilizer NPK 14-10-26 +3MgO + ME. Furthermore, plants fertilized with crystalline fertilizer NPK 9-10-34 + ME showed good results with an average value of the stem length of 112 mm, which is only about 1 mm (0.9 %) lower value in comparison with treatment IV. The lowest average value for the stem length (71 mm) was obtained in the control treatment. The plants fertilized with crystalline fertilizer NPK 9-10-34 + ME had the highest homogeneity (8.1 %) for the length of stem and plants, while plants fertilized with crystalline fertilizer 14-7-28 + 5CaO + M.E. had the most heterogeneous length of stem (CV 15.76 %).

The length of stems that were obtained by applying different crystalline fertilizers showed significant statistical difference at 0.01 level of probability compared with the control treatment. Among the treatments which were fertilized there was no statistically significant difference (Table 2.).

Table 2. Length of stem (mm)

Treatments	Arithmetic mean	Standard error	Standard deviation	Coefficient of variation	Interval of variation	Comparison with Var. 1	Comparison with Var. 2	Comparison with Var. 3	Comparison with Var. 4
I	71	1	9.6	13.55	54-95	Ø	-41	-38	-42
II	112	1	9.1	8.1	90-137	41	Var. 2	3	-1
III	109	1.8	17.2	15.76	79-168	38	-3	Var. 3	-4
IV	113	1.6	15.1	13.37	85-160	42	1	4	Var. 4

LSD 0.05 = 10.94

LSD 0.01 = 15.19

The average length of the root ranges from 140 mm in plants from the control treatment to 215 mm in plants fertilized with crystalline fertilizer NPK 9-10-34 + M.E.. Plants fertilized with crystalline fertilizer NPK 14-10-26 +3 MgO + M.E. showed similar results with plants of treatment II, with an average value of the root length of 214 mm, which is only about 1 mm (1 %) lower in comparison with treatment II. Plants in all treatments showed homogeneity in terms of the root length (CV <30%). The treatments II, III and IV show statistically significant difference at 0.01 level of probability compared to the control treatment. Among the fertilized treatments there was no statistically significant difference (Table 3).

Table 3. Length of root (mm)

Treatments	Arithmetic mean	Standard error	Standard deviation	Coefficient of variation	Interval of variation	Comparison with Var. 1	Comparison with Var. 2	Comparison with Var. 3	Comparison with Var. 4
I	140	2.4	22.5	16.04	108-210	Ø	-75	-67	-74
II	215	3.7	34.9	16.22	145-323	75	Var. 2	8	1
III	207	2.9	27.7	13.34	74-281	67	-8	Var. 3	-7
IV	214	3.3	31.5	14.73	145-312	74	-1	7	Var. 4

LSD 0.05 = 31.88

LSD 0.01 = 44.24

Thickness ranged from 6.9 to 8.7 mm. The largest average thickness of the stem was acquired with crystalline fertilizer NPK 9-10-34 + M.E., while plants from the control treatment had the lowest average thickness of the stem. For this parameter, plants fertilized with crystalline fertilizer NPK 9-10-34 + ME show the highest homogeneity (10.98 %), while the plants of control treatment had the most heterogeneous thickness of the stem (CV 14.24 %). The thicknesses of stems that were obtained by applying different crystalline fertilizers showed significant statistical difference at 0.01 level of probability compared with the control treatment. Among the fertilized treatments there was no statistically significant difference (Table 4).

Table 4. Thick of stem (mm)

Treatments	Arithmetic mean	Standard error	Standard deviation	Coefficient of variation	Interval of variation	Comparison with Var. 1	Comparison with Var. 2	Comparison with Var. 3	Comparison with Var. 4
I	6.9	0.1	0.98	14.24	5-8.9	Ø	-1.8	-1.3	-1.6
II	8.7	0.1	0.95	10.98	6.9-10.9	1.8	Var. 2	0.5	0.2
III	8.2	0.11	1.07	13.13	6.5-8.2	1.3	-0.5	Var. 3	-0.3
IV	8.5	0.11	1.0	11.85	6.8-10.7	1.6	-0.2	0.3	Var. 4

LSD 0.05 = 0.62

LSD 0.01 = 0.87

The highest average value for the mass of plant (50.60 g) was obtained in plants fertilized with crystalline fertilizer NPK 14-10-26 + 3MgO + ME. Furthermore, plants fertilized with crystalline fertilizer NPK 9-10-34 + ME showed good results with an average value of the mass of the plant (50.12 g), which is about 0.48 g (1 %) lower in comparison with treatment IV. The lowest average value for the plant mass (25.06 g) was obtained in the control treatment. Plants fertilized with crystalline fertilizer NPK 14-7-28 + 5CaO + M.E. had the highest homogeneity (11.72 %) for the plant mass, while the plants of control treatment showed the most heterogeneous plant mass (CV 16.43 %). The treatment I, II and III showed statistical significant difference at 0.01 level of probability compared with the mass of plant from the control treatment. Among the fertilized treatments there was no statistically significant difference (Table 5).

Table 5. Mass of plant (g)

Treatments	Arithmetic mean	Standard error	Standard deviation	Coefficient of variation	Interval of variation	Comparison with Var. 1	Comparison with Var. 2	Comparison with Var. 3	Comparison with Var. 4
I	25.06	0.43	4.12	16.43	16.9-35.7	Ø	-25.06	-23.02	-25.54
II	50.12	0.71	6.78	13.53	39.5-68.1	25.06	Var. 2	2.04	-0.48
III	48.08	0.59	5.63	11.72	37.9-61.9	23.02	-2.04	Var. 3	-2.52
IV	50.60	0.73	6.96	13.75	35.9-74.3	25.54	0.48	2.52	Var. 4

LSD 0.05 = 4.05

LSD 0.01 = 5.61

The number of branches was highest in the plants fertilized with crystalline fertilizer NPK 14-10-26 +3 MgO + ME. Also, they show the highest homogeneity (21.21 %) for the number of branches. Plants of control treatment had the lowest number of branches (in averaged 2.2 branches). These plants are most heterogeneous (CV 30.75%). Plants that were fertilized with different crystalline fertilizers showed statistically significant difference in the number of branches compared with control treatment. Among the fertilized treatments there was no statistically significant difference (Table 6.).

Table 6. Number of branches

Treatments	Arithmetic mean	Standard error	Standard deviation	Coefficient of variation	Interval of variation	Comparison n with Var. 1	Comparison n with Var. 2	Comparison n with Var. 3	Comparison n with Var. 4
I	2.2	0.07	0.68	30.75	1-3	Ø	-2.83	-2.31	-3.12
II	5.1	0.12	1.17	23.23	3-7	2.83	Var. 2	0.52	-0.29
III	4.5	0.1	0.96	21.23	3-7	2.31	-0.52	Var. 3	-0.81
IV	5.3	0.12	1.13	21.21	3-8	3.12	0.29	0.81	Var. 4

LSD 0.05 = 0.61

LSD 0.01 = 0.85

The number of inflorescences per plant ranged from 1 to 3. The highest average number of inflorescences (2.1) was obtained in plants that were fertilized with crystalline fertilizer NPK 9-10-34 + ME and NPK 14-10-26 +3 MgO + M.E. Plants fertilized with crystalline fertilizer NPK 14-7-28 +5 CaO + ME gave approximately the same results with previous ones, with an average value of 2 inflorescences. Plants from the control treatment had the lowest values, with an average value of 1.7 inflorescences. These plants were most heterogeneous regarding the parameter (CV 26.64%). The treatment I, II and III showed statistically significant difference compared with plants of the control treatments. Among the fertilized treatments there was no statistically significant difference in terms of the number of inflorescences.

Table 7. Number of inflorescences

Treatments	Arithmetic mean	Standard error	Standard deviation	Coefficient of variation	Interval of variation	Comparison with Var. 1	Comparison with Var. 2	Comparison with Var. 3	Comparison with Var. 4
I	1.7	0.05	0.46	26.64	1-2	Ø	-0.38	-0.30	-0.43
II	2.1	0.04	0.39	18.5	1-3	0.38	Var. 2	0.08	-0.06
III	2	0.03	0.28	13.93	1-3	0.30	-0.08	Var. 3	-0.13
IV	2.1	0.04	0.38	17.91	1-3	0.43	0.06	0.13	Var. 4

LSD 0.05 = 0.18

LSD 0.01 = 0.25

Conclusion

The analysis of the biometric parameters which determined the quality of seedlings of *Pelargonium hortorum* LH Bail., showed that the best results for length of the stem, mass of the plant and number of branches were obtained in fertilized plants with crystalline fertilizer NPK 14-10-26 +3 MgO + M.E..

The best results for length of the root and thickness of the stem showed treatment with crystalline fertilizer NPK 9-10-34 + M.E.

The highest average number of inflorescences (2.1) was obtained from plants that were fertilized with crystalline fertilizer NPK 9-10-34 + ME and NPK 14-10-26 +3 MgO + M.E. The analysis of the parameters which determine the quality of seedlings which are fertilized with different crystalline fertilizer showed that fertilizer type has influence on the quality of seedlings. Quality seedlings can be obtained only by the application of appropriate fertilizers and application of appropriate technology for production of certain plant species.

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UDC: 635.25(497.7)
Original scientific paper**DRY MATTER, SOLUBLE SOLIDS AND ASH CONTENT IN ONION
BUCHINSKA ARSHLAMA DURING STORAGE IN R. MACEDONIA**Bogevska Z.^{1*}, Agic R.¹, Popsimonova G.¹, Davitkovska M.¹, Iljovski I.¹¹Faculty of Agricultural Sciences and Food - Skopje, Ss. Cyril and Methodius University in
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Abstract

Preserving quality of onion bulbs during long term storage depends on storage facilities. In Macedonia, sweet onion landrace '*buchinska arshlama*' is traditionally stored from October to April. During storage there are losses of physiological and chemical nature. The objective of this research was to determine total dry matter, soluble solids and ash content in onion bulbs during storage period on traditional way and in cold room. The research was done three years (2010/2011, 2011/2012 and 2012/2013) and the storage period was from October to April. Every month samples were taken for chemical analysis and they were done according "Rulebook for carrying out the method for chemical and physical analysis due to quality control of fruit and vegetable products". The results were statistically processed and tested with t-test.

There weren't many differences in values of chemical analysis during storage of '*buchinska arshlama*' on traditional way and in the cold room. Total dry matter and soluble solids content was slightly lower in bulbs that were stored in cold room, except in 2012/2013, when the average content of dry matter and soluble solids was significantly higher in the bulbs that were traditionally stored (9.55%/8.78% and 8.72/8.20 respectively). Average values of ash content didn't differ significantly in the examined years for traditional and cold room storage where the biggest difference was registered in 2010/2011 (0.343 and 0.358 respectively).

Key words: onion, storage, dry matter, soluble solids, ash.

Introduction

The onion is an important vegetable crop in our country and is commonly grown by seedlings and onion sets. Summer types of onions called '*arshlami*' ('*buchinska*' and '*gostivarska arshlama*') are grown from seedlings and are intended to be stored longer period.

Buchinskata arshlama, which is mostly cultivated in Pelagonija, is late landrace. The harvest is in the middle of September and is usually stored from October to April. It is sweet type of onion as the total dry matter content varies from 8 to 11% (Simonov D., 1990). In relation to others sweet types of onion this landrace is well stored during winter period (Simonov D., 1980).

The traditional way of storage is in piles covered with plastic films outdoors in the yards of farmers. During the storage there are losses of physiological and chemical nature.

The objective of this research was to determine the total dry matter, soluble solids and ash content during the storage of 'buchinska arshlama' on traditional way and in cold rooms from October to April.

Material and methods

In order to achieve the main objective as material was chosen the local landrace 'buchinska arshlama' grown in the village Vogjani in Pelagonia region.

This type of onion was stored outdoors in the yard of the farmer Igor Veljanoski and in cold rooms of the company „Altra“- Gevgelija. The temperature in the cold room was maintaining from 0 °C to 2 °C and the relative humidity from 90% to 95% during storage period. This experiment was repeated three years (2010-2011, 2011-2012 and 2012-2013), with storage period from October to April with two weeks shelf life at a temperature of 14 to 16 °C for onion bulbs that were stored in cold room .

The chemical analyses were done in the Centre for public health in Veles, R. Macedonia. Every month samples of sound and intact bulbs were taken from the traditional way of storage and cold room. The analyses were done according methods of "Rulebook for carrying out the method for chemical and physical analysis due to quality control of fruit and vegetable products" published in the Official Gazette of the SFRY no. 29/83.

To monitor quality changes in chemical matters these parameters were measured:

- Total dry matter % (drying in the dryer at 105°C);
- Soluble solids % (by refractometer);
- Ash % (direct burning at 600°C).

The statistics of the data obtained during the survey was done with statistical program SPSS for Windows 13,0. The analyzed parameters are displayed with descriptive statistics or mean values and standard deviation (SD). To test the significance of differences between the analyzed parameters were used Analysis of Variance and Student's t-test. For the level of significance a value of $p < 0,05$ was taken but for a high significance was taken a value of $p < 0,01$.

Results and discussion

In this research, regarding the content of total dry matter, the highest average value of 9.63% was achieved in 2010/2011, and the lowest value of 9.53% in 2011/2012 in traditional way of storage. The statistical analysis determined insignificant difference ($p = 0,92$) compared to the average values of total dry matter in the traditional way of storage. The year of examination didn't have significant impact on the average values of the percentage of total dry matter (Table 1).

SECTION 5. VEGETABLE, FLOWER AND DECORATIVE PLANTS PRODUCTION

Table 1. Total dry matter content in bulbs stored in a traditional way in %

Period of measurement	2010/2011	2011/2012	2012/2013
Harvest	9,00	8,50	9,50
October	9,30	8,70	9,70
November	9,50	10,40	9,80
December	9,40	9,50	9,33
January	9,70	10,00	9,27
February	10,00	9,65	9,53
March	9,62	9,30	9,80
April	10,49	10,20	9,47
mean±SD	9,63±0,46	9,53±0,68	9,55±0,20
2010/2011 – 2011/2012 – 2012/2013 -	Analysis of Variance F=0,08 p=0,92		

In cold room storage highest average value of total dry matter of 9.43% was achieved in 2011/2012, while the lowest average value of 8.79% was set out in 2012/2013. It was found that there weren't significant differences ($p = 0,058$) among the average values for the content of total dry matter in the bulbs that were stored in the cold room in three years research (Table 2).

Table 2. Total dry matter content in bulbs stored in cold room %

Period of measurement	2010/2011	2011/2012	2012/2013
Harvest	9,00	8,50	9,50
October	9,30	8,70	9,70
November	9,20	10,0	7,63
December	9,30	9,30	8,87
January	9,06	9,80	8,00
February	9,50	10,2	7,80
March	10,00	9,10	9,50
April	9,90	9,88	9,17
Shelf life 2 weeks on 14°C-16°C	9,30	9,30	8,90
mean±SD	9,396±0,35	9,43±0,6	8,79±0,79
2010/2011 – 2011/2012 – 2012/2013	Analysis of Variance F=3,22 p=0,058		

The content of total dry matter in the three years of research from harvest until April, or after the shelf life procedure in 2010/2011 amounted 9.63% for bulbs stored traditionally while in cold room the content was insignificantly less and amounted 9.39%. In 2011/2012, the content of total dry matter amounted 9.50% for bulbs that were stored traditionally and less but not significant of 9.43% for bulbs stored in cold room. In 2012/2013, the average value of total dry matter varies significantly depending on the way of storage ($p = 0,018$), as a result of significantly higher value of the content of total dry matter in the bulbs that were traditionally stored ($9,55 \pm 0,2\%$ and $8,78 \pm 0,79\%$ respectively) (Table 3).

SECTION 5. VEGETABLE, FLOWER AND DECORATIVE PLANTS PRODUCTION

Table 3. Average values for the content of total dry matter in the bulbs that were stored traditionally and in cold room

Period of storage	Traditional storage mean±SD	Cold room storage mean±SD	t-test	p-value
2010/2011	9,63±0,46	9,39±0,35	1,18	0,26
2011/2012	9,50±0,68	9,43±0,6	0,32	0,60
2012/2013	9,55±0,2	8,78±0,79	2,66	0,018*

*p<0,05

Similar results (from 7.88% to 9.99%) in terms of the content of total dry matter in *'buchinska arshlama'* found Simon D., 1980. Kimani P.M. et al. (1993) examined 8 foreign and 3 local varieties of onion where the total dry matter content was ranging from 7% to 10% and from 15% to 20% depending on variety. The varieties *'red synthetic'* and *'red creole'* showed similar values in the content of total dry matter as *'buchinska arshlama'* (9.1% and 10.1% respectively). According Kukanoor L. (2005), increasing total dry matter content during storage could be attributed to the increase of chemical compounds, and also of reducing the moisture content of the bulbs. In this research, excluding the year 2012/2013, total dry matter content in traditional and cold room storage slightly increased in comparison with the content at the beginning of storage as a result of water loss of the bulbs during the storage. These results are in line with previously cited author. The average values for the soluble solids content in onion bulbs that were stored traditionally amounted 8.89% in 2010/2011, 8.94% in 2011/2012 and 8.72% in 2012/2013. There weren't significant differences in relation to the average values for soluble solids content in bulbs that were stored on traditional way depending on the year (Table 4).

Table 4. Soluble solids content in bulbs that were stored on traditional way in %

Period of measurement	2010/2011	2011/2012	2012/2013
Harvest	8,50	7,50	9,20
October	8,70	8,25	9,35
November	9,10	9,90	9,20
December	8,90	9,10	8,18
January	9,00	9,60	8,20
February	9,60	8,90	8,38
March	8,50	9,00	9,00
April	8,80	9,30	8,28
mean±SD	8,89±0,36	8,94±0,76	8,72±0,51
2010/2011 – 2011/2012 – 2012/2013	Analysis of Variance F=0,32 p=0,73		

The year of research also didn't have significant impact on the average values of the soluble solids content in bulbs that were stored in cold room (p = 0,25). The average values of soluble solids content in bulbs stored in cold room were 8,79 ± 0,3% in 2010/2011, 8,68 ± 0,64% in 2011/2012 and 8,2 ± 1,1% in 2012/2013 (Table 5).

Table 5. Soluble solids content in bulbs that were stored in cold room in %

Period of measurement	2010/2011	2011/2012	2012/2013
Harvest	8,50	7,50	9,20
October	8,70	8,20	9,30
November	8,60	9,70	6,60
December	8,80	8,90	7,20
January	8,40	9,30	7,30
February	8,80	8,60	7,20
March	9,40	8,70	9,10
April	8,90	8,30	8,90
Shelf life 2 weeks on 14°C-16°C	9,00	8,90	9,10
mean±SD	8,79±0,3	8,68±0,64	8,2±1,1
2010/2011 – 2011/2012 – 2012/2013	Analysis of Variance F=1,48 p=0,25		

There weren't significant differences between average values of soluble solids content in bulbs that were stored on traditional way and in cold room except in 2012/2013 where the average value of soluble solids content in bulbs that were traditionally stored was significantly higher ($p = 0,025$) than bulbs that were stored in cold room (Table 6).

Table 6. Tested differences in average values of soluble solids content in bulbs that were stored traditionally and in cold room

Period of storage	Traditional storage mean±SD	Cold room storage mean±SD	t-test	p-value
2010/2011	8,89±0,36	8,79±0,30	0,62	0,55
2011/2012	8,94±0,76	8,68±0,64	0,78	0,45
2012/2013	8,72±0,51	8,20±1,1	1,2	0,025*

* $p < 0,05$

Similar values (from 7.50% to 9.00%) regarding the soluble solids content in bulbs of 'buchinska arshlama' found Simon D. (1980).

The ash content in the onion bulb range from 0,3% to 0,7% (Lazić B. *et al.*, 1998; Lawande K.E., 2001). In this research the minimum and the maximum ash content range from 0.30% to 0.45% in all examined years in both storage facilities, so it can be concluded that the values match the reference data. The highest average value in relation to ash content in traditional way of storage was found in 2011/2012 (0.372%), but lowest in 2010/2011 (0.343%). The differences in average values of ash content between the analyzed period were statistically insignificant ($p = 0,37$) (Table 7).

SECTION 5. VEGETABLE, FLOWER AND DECORATIVE PLANTS PRODUCTION

Table 7. Ash content in bulbs that were stored on traditional way in %

Period of measurement	2010/2011	2011/2012	2012/2013
Harvest	0,35	0,30	0,40
October	0,32	0,35	0,39
November	0,34	0,39	0,34
December	0,36	0,39	0,33
January	0,30	0,33	0,31
February	0,43	0,44	0,34
March	0,30	0,38	0,40
April	0,35	0,40	0,44
mean±SD	0,343±0,042	0,372±0,044	0,369±0,045
2010/2011 – 2011/2012 – 2012/2013	Analysis of Variance F=1,03 p=0,37		

The average values for ash content didn't differ significantly between the three years research in bulbs that were stored in cold room ($p = 0,44$). Average values for the ash content amounted $0,358 \pm 0,022\%$ in 2010/2011, $0,384 \pm 0,06\%$ in 2011/2012 and $0,367 \pm 0,041\%$ in 2012/2013 (Table 8).

Table 8. Ash content in bulbs stored in cold room in %

Period of measurement	2010/2011	2011/2012	2012/2013
Harvest	0,35	0,30	0,40
October	0,32	0,35	0,39
November	0,34	0,33	0,31
December	0,35	0,45	0,33
January	0,37	0,32	0,33
February	0,36	0,41	0,38
March	0,38	0,46	0,33
April	0,37	0,44	0,41
Shelf life 2 weeks on 14°C-16°C	0,39	0,40	0,42
mean±SD	0,358±0,022	0,384±0,06	0,367±0,041
2010/2011 – 2011/2012 – 2012/2013	Analysis of Variance F=0,84 p=0,44		

Average values for the ash content in onion bulbs in both ways of storage didn't differ significantly during examined period. The biggest difference in the average ash content was registered in 2010/2011 ($0,343 \pm 0,042$ and $0,358 \pm 0,02$ respectively), but it wasn't sufficient for statistical significance (Table 9).

Table 9. Tested differences in average values of ash content in bulbs that were stored traditionally and in cold room

Period of storage	Traditional storage mean±SD	Cold room storage mean±SD	t-test	p-value
2010/2011	0,343±0,042	0,358±0,022	1,47	0,15
2011/2012	0,372±0,044	0,384±0,06	0,46	0,65
2012/2013	0,369±0,045	0,367±0,041	0,01	0,92

Conclusion

Based on three years research of storing the onion 'buchinska arshlama' by traditional way and in cold room, the following conclusions about chemical matters content can be specified:

- Total dry matter and soluble solids content was slightly insignificant lower in bulbs that were stored in cold room except in 2012/2013, when the average content of dry matter and soluble solids was significantly higher on level 0.5 in the bulbs that were traditionally stored.
- Among bulbs that were traditionally stored and in the cold room there weren't significant differences in the average values of ash content during the examined period.
- Having in mind the losses of weigh, sprouting and rotting which were previously published (Bogevska Z. *et al.*, 2014) and the changes of chemical composition in onion bulbs of 'buchinska arshlama' can be concluded that this onion landrace can be successfully stored traditionally from October to early February and then in controlled conditions (cold room) until April or longer.

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UDC: 635.34.037"321"
Original scientific paper

SPRING CABBAGE TRAITS IN RELATION TO DIFFERENT SEEDLING GROWING TECHNOLOGIES

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Abstract

The production of quality seedlings is one of the key factors for successful vegetable crop production. In the recent years the production of seedlings in warm beds was significantly reduced while the production of seedlings in containers of polystyrene has greatly increased. The production of seedlings in containers facilitates the transplanting of the seedlings and allows controlling of each plant in terms of irrigation, fertilization and protection. Because of these advantages of this method for seedling production, the goal of this research is aimed at identifying and determining the effect on the parameters of the heads and yield of spring cabbage from the modern mode of production of seedlings in containers with three different volume of the cells (var. 1- 24.43cm³; var. 2- 45.8cm³; var. 3-110 cm³), compared to the traditional mode of production in beds. As material for this research was used spring cabbage (*Brassica oleracea* L. var. *capitata*), hybrid Nozomi. To determine the effect of the volume of the cells on the growth of the cabbage were analyzed the following parameters: height of the root and root neck, which ranged from 5,00 cm in the control to 6,98 cm in the variant 1; height of the heads which ranged from 11,71 cm in the variant 3 to 13,81 cm in variant 1; width of the heads which ranged from 13,78 cm in the variant 3 to 16,93 cm in variant 1, weight of heads which ranged from 0,7 kg in the control to 1,0 kg in the variant 2 and yield of heads from 35,1 t/ha in the control to 48,8 t/ha in variant 2.

Key words: spring cabbage, container cells, seedlings, different volume, yield.

Introduction

Many vegetables during their development stages are going through seedling period. Seedlings are young plants in early stages of growth and development (Simonov D. and Aladjakov L., 1985, Tudzarov T., 2011, Lazić B., 2011). They are mainly grown in protected houses (for early and middle production) while the late production is with seedlings grown in open beds (Lazić B. *et al.*, 1998).

In recent years late production with seedlings in warm beds is reduced significantly while the container production is growing. Seedlings production in containers facilitates all required measures as irrigation, fertilization, protection, transplanting (Lešićet *al.*, 2002). For early spring seedling production using containers is the best as plants are transplanted without damaging the roots and without stress.

Head cabbage (*Brassica oleracea* L. var. *capitata*) is one of the most important vegetable which is characterized by long tradition of production in the world and in our republic. Cabbage can be grown with direct sowing and with seedlings (Aladjajkov L., 1966, Popović M., 1973, Đinović I., 1998). The advantage of the seedling production of cabbage is short vegetation, less seeds and easier care in earlier stages of plant growth and development. Seedling production of cabbage in our country is on traditional way in beds but today increasingly is applied modern methods of production as containers. Therefore the objective of this research is to identify and determine the effect of the modern way of seedling production in containers with different cells volume compared to the traditional production in beds on yield components of spring cabbage.

Materials and methods

In this research as material was used early cabbage (*Brassica oleracea* L. var. *capitata*), hybrid Nozomi F1. The hybrid Nozomi F1 is early cabbage hybrid which has round head and is mainly used for fresh consumption. It has uniform head and resistance to cracking and rot. The vegetation period last 55-60 days. The head has dark green colour and is used for open and protected production.

For this purpose it was used containers with different volume of cells and as control was used production in warm beds.

Sowing date was on 12.12.2010. The seeds sprouted in 4 days after sowing at 18-20 °C. Transplanting of the seedlings was after 48 days after sowing.

The containers used for seedling production were with dimensions 28 x 50 cm, but had different number of cells with different size and volume.

Experiment was set in a randomized block design with four variants, including control with three repetitions. The variants were set depending on the volume of container cells and used as a control warm bed. Arrange of variants was as follows:

1. Ø Control-sowing cabbage in warm bed;
2. Variant 1- sowing cabbage in containers with cell volumes of 24,43 cm³/0,024 L;
3. Variant 2 - sowing cabbage in containers with cell volumes of 45,8cm³/0,046L;
4. Variant3 - sowing cabbage in containers with cell volumes of 110 cm³/0,11L.

The substrate used for containers was PC1(Agro CS) with standard package of 250 L. The fraction of the substrate was from 0 to 20 mm and was made of 70% white peat and 30% of black peat enriched with nitrogen (100-250mg/L), phosphorus (100-250mg/L), potassium (150-300mg/L) with conductivity of 1 (EC = 1) and pH value from 5.5 to 6.5.

In order to determine the effect of the cells volume on growth and yield of cabbage following parameters were investigated:

- Height of the root and root neck (cm)
- Height of the heads (cm)
- Width of the heads (cm)
- Weight of heads (kg)
- Yield of heads (t/ha)

All data from the survey were statistically processed using analysis of variance (ANOVA), while the significance of differences was determined by LSD test.

Results and discussion

The root of the cabbage is densely overgrown with root hairs, but in general is poorly developed in comparison to the above part of the plant.

Table 1. Height of the root and root neck (cm)

Repetition	Control Ø	Variant 1 (28)	Variant 2 (66)	Variant 3 (160)
I	5,1	6,7	6,2	6,7
II	5,3	6,9	5,8	6,7
III	4,7	7,2	5,8	6,9
Mean	5,00	6,93	5,93	6,78
+ - from Ø	Ø	1,9**	0,9**	1,8**
Standard deviation (σ)	0,30	0,27	0,26	0,15
Coefficient of variation (cv)	6,09	3,96	4,33	2,14
Standard error of a mean	0,22	0,19	0,18	0,10
LSD _{0,05} *	0,359			
LSD _{0,01} **	0,559			

According to the results of the height of the root and root neck (Table 1) the highest average value was achieved in the variant 1 (6,93cm), while the lowest value in control (5,00 cm). The highest coefficient of variation was determined in control (6.09%), followed by variant 2 (4.33%), then variant 1 (3.69%), and the lowest coefficient of variation was determined in variant 3 (2.14%). According to Červenski et al., (2003), average height of the cabbage head is about 18,9cm. Results of cabbage head height showed that the variant 2 had the highest average value of cabbage head height (16,91cm), while the lowest the variant 3 (11,71cm).

The coefficient of variation was highest in control (2,34%), then in variant 1 (1,81%), following the variant 2 (1,40), while the lowest coefficient of variation was obtained in variant 3 (0,86). There were statistically significant differences between control and all treatments on the two levels (0,05 and 0,01). The width of the cabbage head is about 22,6cm (Červenski, 2010).

Table 2. Height of the head (cm)

Repetition	Control Ø	Variant 1 (28)	Variant 2 (66)	Variant 3 (160)
I	13,1	13,6	17,2	11,7
II	13,3	13,8	16,8	11,6
III	12,7	14,1	16,8	11,8
Mean	13,00	13,81	16,91	11,71
+ - from Ø	Ø	0,8**	3,9**	-1,3**
Standard deviation (σ)	0,30	0,25	0,24	0,10
Coefficient of variation (cv)	2,34	1,81	1,40	0,86
Standard error of a mean	0,22	0,18	0,17	0,07
LSD _{0,05} *	0,356			
LSD _{0,01} **	0,555			

According to the measurements that referred to the width of the early cabbage head the highest average value for this parameter was obtained in variant 1 (16,93 cm), while the lowest average value was found in variant 3 (13,78 cm).

The coefficient of variation was highest in control (2,84%), while the lowest coefficient of variation was obtained in variant 3 (1,05%).

Regarding the width of the cabbage head after statistical data processing there were obtained statistically significant differences between the control variant and variants 1 and 2 on the two levels of significance 0,05 and 0,01.

Table 3. Width of the head (cm)

Repetition	Control Ø	Variant 1 (28)	Variant 2 (66)	Variant 3 (160)
I	14,1	16,7	15,2	13,7
II	14,3	16,9	14,8	13,7
III	13,5	17,2	14,8	13,9
Mean	13,94	16,93	14,93	13,78
+ - from Ø	Ø	3,0**	1,0**	-0,2
Standard deviation (σ)	0,40	0,27	0,26	0,15
Coefficient of variation (cv)	2,84	1,62	1,72	1,05
Standard error of a mean	0,28	0,19	0,18	0,10
LSD _{0,05} *	0,418			
LSD _{0,01} **	0,651			

When you remove outer leaves and stem remains the useful part of the cabbage the head. According to Červenski, (2010), the mature cabbage heads weigh from 1,4 to 2,3 kg, sometimes more, which depends on variety, production conditions and used agro-technical measures.

In the following table 4 are given the values of cabbage head weight.

Table 4. Weight of the head (kg)

Repetition	Control Ø	Variant 1 (28)	Variant 2 (66)	Variant 3 (160)
I	0,8	0,9	0,9	0,8
II	0,7	0,9	1,0	0,7
III	0,6	1,0	1,0	0,8
Mean	0,7	0,9	1,0	0,8
+ - from Ø	Ø	0,2**	0,3**	0,1
Standard deviation (σ)	0,13	0,06	0,04	0,06
Coefficient of variation (cv)	18,42	6,66	4,49	8,41
Standard error of a mean	0,09	0,04	0,03	0,05
LSD _{0,05} *	0,107			
LSD _{0,01} **	0,167			

According the results given in the table 4 the highest average value for head weight was obtained in variant 2 (1,0 kg), which is 0,3 kg higher in comparison with the control variant (0,7 kg). Higher average value of cabbage head weight was obtained in variant 1 (0,9 kg), which is 0,2 kg higher than the control and in variant 3 the average cabbage head weight was 0,8 kg, which is for 0,1 kg higher than the control variant.

The highest coefficient of variation was obtained in control (18,42%), while in all variant the coefficient of variation was significantly lower, as follows: 8.41% for variant 3, 6.66 % for variant 1 and 4.49 % for variant 2.

Statistically significant difference at the level of 0.05 and 0,01 was determined between the control and variant 1 and 2.

The harvest of cabbage is performed in technological maturity with cutting the head by hand or by machine. Mechanized harvesting is one time while the hand harvest is 2 to 3 times once heads reached the weight and size typical for the variety. Early cabbage is harvest at the end of May and beginning of June while late cabbage is harvest on or two times at the end of October and beginning of November before frost occur (below -5°C). The yield of early cabbage varieties is 20-30t/ha, of middle early varieties 35-40 t/ha and late cabbage varieties 40-50 t/ha (Lazić *et al.*, 1998).

The results obtained in this research that referred to the yield of heads expressed in t/ha derived from plants produced by seedlings grown on traditional way (warm bed - control) and in containers with different cells volume (variant 1, variant 2, variant 3) are given in table 5.

Table 5. Yields of heads (t/ha)

Repetition	Control Ø	Variant1 (28)	Variant 2 (66)	Variant 3 (160)
I	40,6	43,3	46,6	39,5
II	36,8	43,9	48,8	34,0
III	28,0	48,8	51,0	39,5
Mean	35,1	45,4	48,8	37,7
+ - from Ø	Ø	10,2*	13,7**	2,6
Standard deviation (σ)	6,47	3,02	2,19	3,17
Coefficient of variation (cv)	18,42	6,66	4,49	8,41
Standard error of a mean	4,57	2,14	1,55	2,24
LSD _{0,05} *	8,0			
LSD _{0,01} **	11,8			

The highest average yield was obtained in variant 2 (48,8 t/ha), while the lowest yield was obtained in control variant (35,1 t/ha). Variants 1 and 3 gave higher yields in comparison with control (10,2 t/ha and 2,6 t/ha respectively). Statistically significant difference was determined between control and variant 2 on level 0.01, while between control and variant 1 was determined statistically significant difference on level 0.05.

The highest value for coefficient of variation was obtained in control (18,42%), then in variant 3 (8,41%), following the variant 1 (6,66%), while the lowest coefficient of variation was determined in the variant 2 (4,49%).

Conclusion

According to the results obtained in this research, and the experiences of producers and researchers can be concluded that usage of seedling production in containers is positively justified, especially for early vegetable production, both in terms of convenience in manipulation with seedlings during its production, and in terms of positive impact on the development and quality of plants, which leads to realization of the main goal of producers - getting high and stable yields.

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UDC: 635.25-167(560)"2009/2010"
Original scientific paper**ONION VEGETATIVE GROWTH, YIELD AND QUALITY PARAMETERS
UNDER DRIP AND SUBSURFACE DRIP IRRIGATION METHODS**Erdem T.^{1*}, A. Kayhan¹¹Namık Kemal University, Agricultural Faculty, Tekirdağ-Turkey

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Abstract

The experiment was conducted during the growing season 2009 and 2010 to evaluating the vegetative growth, yield and quality parameters of onion under different irrigation and irrigation water amounts. The irrigation water amounts applied based on a ratio of Class A pan evaporation as 50, 75, 100 and 125 % under drip irrigation and subsurface drip irrigation with 20 cm lateral depth were investigated. The seasonal evapotranspiration in the treatments varied from 337.14 mm to 715.39 mm in 2009 and from 388.79 mm to 579.26 mm in 2010. The 6% less water consumption was averagely measured in subsurface drip irrigation method than drip irrigation method in both years. The total marketable onion yield ranged from 12.73 t ha⁻¹ to 19.44 t ha⁻¹ in 2009 and from 17.82 t ha⁻¹ to 25.66 t ha⁻¹ in 2010 for drip irrigation treatments while it ranged from 15.13 t ha⁻¹ to 25.99 t ha⁻¹ in 2009 and from 22.60 t ha⁻¹ to 32.31 t ha⁻¹ for subsurface drip irrigation treatments. In the research, the plant height and leaves number as vegetative growth parameters, total marketable yield, bulb weight, bulb height and bulb size as yield parameters and dry matter content, soluble solids concentration, pH, protein content, total sugar content, invert sugar content as quality parameters were determined under different irrigation methods and irrigation water amount conditions. In general, there were no uniformly statistical differences in the vegetative growth, yield and quality parameters among the treatments.

Keywords: Irrigation method, evapotranspiration, lateral depth, onion.

Introduction

Production of onion (*Allium cepa* L.) takes a very important place in world agriculture, with a production potential of about 72 million t harvested and 3.8 million ha planted area (FAO, 2009). Onion is one of the main crops in Turkey where the production is about 1.85 million t harvested from 65 000 ha (FAO, 2009). Early studies have shown that water is the most limiting factor for onion production and it is possible to increase production levels by well-scheduled irrigation programs (Şener 1999, Subedi et al., 2002, Kumar et al., 2007, Enciso et al., 2007, Patel and Rajput 2009, Pejic et al., 2011).

Subsurface drip irrigation system has been part of drip irrigation development in USA since 1960, but regular research was started on since early 1980s. Subsurface drip irrigation system is used to provide water to a substantial portion of the plant root system while maintaining a relatively dry soil surface (Camp 2000, Patel and Rajput 2009). Ayars et al., (1999) summarized 15 years of research conducted of row crops in California by

observing that significant amount of water can be saved through subsurface drip irrigation. The previous studies shown that crops were irrigated by surface drip irrigation, yields were equal to or greater than obtained by surface drip irrigation (Machado and Oliveria 2005, Önder et al., 2005, Kalfountzous et al., 2007, Singh and Rajput 2007, Doğan et al., 2008, Patel and Rajput 2009, Lamm et al., 2010).

The objective of this research was to evaluate the effects of surface and subsurface drip irrigation methods and irrigation water level on onion vegetative growth, yield and quality parameters under Tekirdag conditions, Turkey. Also, the feasibility of subsurface drip irrigation has been investigated under Thrace Region, Turkey.

Materials and methods

This study was conducted in 2009 and 2010 growing seasons at the farm at the Karaevli Village, Tekirdag, Turkey (northwestern part of Turkey) at 41°02'N latitude, 27°39'E longitude and 148 m altitude. The climate in the research field is classified as semi-arid and the averages of annual temperature, relative humidity, wind speed, sunshine duration per day and total precipitation are 13.9°C, 78%, 2.7 m s⁻¹, 6.5 h and 585.1 mm. Soil type in the plot area is clay-loam for first year and loam for second year. There are no salinity and alkalinity problems. The bulk density varies from 1.52 g cm⁻³ to 1.55 g cm⁻³ in 2009 and from 1.45 g cm⁻³ to 1.57 g cm⁻³ in 2010. The available water holding capacity within 60 cm of the soil profile is 96.47 mm (2009) and 105.36 mm (2010). Irrigation water quality is classified as C₂S₁ with 0.33 sodium absorption ratio (SAR) and 0.1 dS m⁻¹ electrical conductivity (EC).

Onions were direct seeded on April 10 every two years and harvested in June 24 (2009) and June 20 (2010). Fertilizers, herbicides and insecticides were applied, when necessary, uniformly to each plot when the soil was ploughed. Each plot covered an area of 7.20 m² (2.4 m x 3.0 m) and contained 180 plants 0.20 m x 0.20 m spacing. There was a 3 m space to reduce the edge effect between each plot. Water was taken from deep well and pumped to small reservoir. The plots were irrigated from reservoir by pump. The control unit consisted of screen filter with 25 L s⁻¹ capacity, a pressure regulator and manometers mounted on the inlet and outlet of each unit. Polythene (PE) tube was used for 50 mm main and 16 mm manifolds of drip and subsurface drip irrigation systems. The diameters of the laterals were 16 mm PE, each lateral irrigated two plant rows and the lateral spacing were chosen as 0.40 m. Subsurface drip lateral lines were installed 0.20 m below the soil surface and lateral lines were installed just prior to planting. Pressure compensating drippers were used to supply uniform water distribution. The dripper discharge rate was 1.8 L h⁻¹ above 10 m operating pressure. Dripper spacing was chosen as 0.20 m depending on the soil characteristics. Thus, the percentage of the wetted area (P) that relates dripper spacing to lateral spacing was determined as 50% by the methods described by Keller and Bliesner (1990).

A split-plot design with three replications was used with two irrigation methods (main plots) and four irrigation regimes (sub-plots). The irrigation methods treatments consisted of drip and subsurface drip irrigation methods. The irrigation regime treatments consisted of four levels of cumulative pan evaporation (E_p) and water quantities applied were as 0.50 (I₁), 0.75 (I₂), 1.00 (I₃) and 1.25 (I₄) times of pan evaporation measured at seven days interval by Class A Pan located in the experimental site. The amount of irrigation water was calculated by using the equation given below:

$$I = E_p \times k_{cp} \times P$$

where E_p is the cumulative pan evaporation for the 7 day irrigation interval (mm), k_{cp} is the coefficient of pan evaporation and P is the percentage of wetted area.

Soil water content in the plots was gravimetrically measured every week in the 30 cm depth increments to 0.90 m, using by the hand sampler. Evapotranspiration was estimated using the soil water balance equation (Allen *et al.*, 1998). The equation can be written as:

$$ET = I + P \pm \Delta SW - DP - RO$$

where ET is the evapotranspiration (mm), I is the irrigation water (mm), P is the precipitation (mm), ΔSW is the change in the soil water storage in the 0.90 m soil profile (mm), DP is the deep percolation (mm) and RO is the amount of runoff (mm). Since the amount of irrigation water was controlled, run off was assumed to be zero.

The plant height and leaves number per plant were measured for vegetative parameters while total marketable yield, bulb size, bulb weight and bulb height for yield parameters were determined 10 randomly selected plants in each plot. The quality parameters as dry matter content, soluble solids concentration, pH, protein content, total sugar content, invest sugar content were analyzed. Data were analyzed using of variance (ANOVA). The least significant test (LSD) was used to compare and rank treatments (Gomez and Gomez, 1984). Differences were declared significant at $p < 0.05$ or 0.01 .

Results and discussion

The amount of total irrigation water, rainfall and measured seasonal evapotranspiration for all treatments during the experimental years are presented Table 1. Rainfall during the total growing period was 33.30 mm in 2009 and 102.70 mm in 2010. The first irrigation as 15 mm on April 10 for each year applied equally to all treatments to achieve good plant germination. In 2009, a total of 194.8 mm, 284.5 mm, 374.1 mm and 464.0 of irrigation water with twelve times were applied to I_1 , I_2 , I_3 and I_4 treatments, respectively, while 145.5 mm, 210.8 mm, 276.0 mm and 341.3 mm irrigation water with seven different times were applied in 2010. Because of the excess rainfall, the applied irrigation water in 2010 was lower than in 2009. Total seasonal evapotranspiration by onion varied from 337.14 mm to 715.39 mm in 2009 and from 388.79 mm to 579.26 mm. The lower seasonal evapotranspiration values in 2010 were generally due to the higher rainfall and lower evaporation during the growing season. Considering the previous studies, the obtained seasonal evapotranspiration values are similar to that summarized as between 217 and 607 mm by Şener (1999), Kumar *et al.*, (2007), Patel and Rajput (2009). Among the irrigation methods, subsurface drip irrigation method caused lower seasonal evapotranspiration compared to drip irrigation method in both years. It was meanly measured as 559.71 mm in 2009 and 501.21 mm in 2010 for drip irrigation method treatments while it was measured as 524.66 mm in 2009 and 471.37 mm in 2010 for subsurface drip irrigation method treatments. In two growing seasons, seasonal evapotranspiration values increased with increasing k_{cp} coefficient. The seasonal evapotranspiration was measured from I_3 treatment (k_{cp} = 100%) as 576.31 mm in 2009 and 529.33 mm in 2010.

The leaves number, plant height, bulb weight, bulb size, bulb height and total marketable yield of onion as vegetative growth and yield parameters obtained from each treatment and summary statistics are presented in Table 2. The effects of irrigation methods, irrigation level and the interaction between the irrigation method and irrigation level on vegetative parameters were not generally significantly different for two year while the effects of

irrigation level on leaves number were statistically significant at the 0.05 confidence level at second year.

Table 1. Applied irrigation water and measured seasonal evapotranspiration for treatments

Year	Irrigation method	Irrigation regimes	Soil water depletion mm	Rainfall mm	Irrigation water use mm	Seasonal evapotranspiration mm 60 cm ⁻¹
2009	Drip	I ₁	205.45	33.30	194.8	433.55
		I ₂	190.85		284.5	508.65
		I ₃	173.85		374.1	581.25
		I ₄	218.09		464.0	715.39
	Subsurface drip	I ₁	109.04		194.8	337.14
		I ₂	177.51		284.5	495.31
		I ₃	163.96		374.1	571.36
		I ₄	197.51		464.0	694.81
2010	Drip	I ₁	155.88	102.70	145.5	404.08
		I ₂	156.94		210.8	470.44
		I ₃	172.34		276.0	551.04
		I ₄	135.26		341.3	579.26
	Subsurface drip	I ₁	140.59		145.5	388.79
		I ₂	106.15		210.8	419.65
		I ₃	128.91		276.0	507.61
		I ₄	125.43		341.3	569.43

The bulb weight, bulb size and bulb height for treatments ranged from 50.93 g to 129.22 g, from 47.93 mm to 64.15 mm and from 51.71 mm to 65.39 mm, respectively. The effects of irrigation method and the interaction between the irrigation method and irrigation on bulb weight, bulb size and bulb height were not statistically significant in both years. However, according to statistical analyses, the irrigation level had effected on bulb weight and bulb height in 2009. The highest bulb weight and bulb height was obtained at I₃ level in 2009. Similar results have been obtained in the previous research (Arın 1993; Şener 1999).

Total marketable onion yield ranged from 12.73 t ha⁻¹ to 25.99 t ha⁻¹ in 2009 and from 17.82 t ha⁻¹ to 32.31 t ha⁻¹ in 2010. These average marketable yield differences between years can be attributed the climatic conditions. Similar results on same climatic conditions and onion varieties have been obtained. Arın (1993) also found that total marketable onion yields at rates of 25.09 - 33.53 t ha⁻¹ while Şener (1999) found that it at rates of 17.29 – 43.07 t ha⁻¹. The highest total marketable onion yields at rates of 20.13 – 27.26 t ha⁻¹ were obtained from subsurface drip irrigation in both years while it was not significant affected by irrigation methods in the both years. Among the irrigation levels, the highest total marketable onion yields in both years occurred at I₄ level ($k_{cp}= 125\%$) with 21.96 t ha⁻¹ (2009) and 27.26 t ha⁻¹ (2010). Also, the total marketable onion yield increased with increasing irrigation water. The total marketable onion yield was only affected by irrigation levels in 2009 according to the variance analysis at confidence level of 1%.

Table 3 show the data on quality parameters as dry matter content, soluble solids concentration, pH, protein content, total sugar content and invert sugar content. The dry matter content ranged from 12.65% to 15.04% in 2009 and from 10.62% to 18.70% in 2010. The influence of irrigation methods, irrigation levels and interaction between the irrigation method and irrigation level on dry matter content was not significant in 2009 while it was significant in 2010. The highest dry matter content was obtained from I₃ ($k_{cp}=$

100%) irrigation level under subsurface drip irrigation in 2010. The soluble solid concentration was not statistically significant at second treatment year while irrigation level only affected it at the 0.05 confidence level at second year in 2009. Among the irrigation level, the highest soluble solid concentration was recorded in I_4 level ($k_{cp}=125\%$) with 12.24 %. Similar results on soluble solid concentration have been obtained in the previous research (Arın 1993 and Şener 1999).

The pH value ranged from 5.45 to 5.61 in 2009 and from 5.63 to 5.77 in 2010. The pH value differences for all treatments were not statistically significant at the 0.05 and 0.01 confidence level. The effects of irrigation methods, irrigation levels and interaction between the irrigation method and irrigation level on protein content was not statistically significant in 2010. Among the irrigation level, the protein content differences in 2009 were statistically significant in 2009 and the highest protein content in 2009 occurred at I_2 level ($k_{cp}=75\%$) with 1.70%. Also, the interaction between the irrigation method and irrigation level on protein content was also statistically significant in 2010. The total sugar content ranged from 8.66% to 13.17% in 2009 and from 9.28% to 13.29% in 2010 while invert sugar content ranged from 1.83% to 2.79 in 2009 and from 1.83% to 4.84 in 2010. According to statistical analyses, interaction between the irrigation method and irrigation level on invert sugar content at confidence level of 5% was only recorded.

SECTION 5. VEGETABLE, FLOWER AND DECORATIVE PLANTS PRODUCTION

Table 2.The onion vegetative growth and yield parameters

Year	Irrigation method	Irrigation regimes	Leaves number	Plant height cm	Bulb weight g	Bulb size mm	Bulb height mm	Total marketable yield t ha ⁻¹
2009	Drip	I ₁	6	42.32	50.93	47.93	51.71	12.73
		I ₂	6	45.62	64.22	51.85	54.75	16.06
		I ₃	6	40.97	77.78	54.08	60.78	19.44
		I ₄	6	43.54	74.72	53.67	58.41	18.68
	Subsurface drip	I ₁	5	39.97	60.54	48.12	52.38	15.13
		I ₂	6	40.47	71.23	50.90	54.85	17.81
		I ₃	6	44.53	86.26	56.86	59.62	21.57
		I ₄	7	46.85	103.98	59.77	65.39	25.99
	LSD		ns	ns	ns	ns	ns	ns
	Drip		6	43.11	66.91	51.88	56.41	16.73
	Subsurface drip		6	42.95	80.50	53.91	58.06	20.13
	LSD		ns	ns	ns	ns	ns	ns
		I ₁	5	41.14	55.74 b	48.02	52.05 c	13.93 b
		I ₂	6	43.04	67.73 ab	51.37	54.80 bc	16.94 ab
		I ₃	6	42.75	82.02 a	55.47	60.20 ab	20.50 a
		I ₄	7	45.19	89.35 a	56.72	61.90 a	22.34 a
		LSD	ns	ns	24.40**	ns	6.804*	6.10**
2010	Drip	I ₁	5	34.53	71.25	51.15	56.28	17.82
		I ₂	6	36.60	82.84	56.53	59.02	20.71
		I ₃	7	37.87	94.48	57.37	56.33	23.62
		I ₄	7	39.40	102.63	62.12	53.28	25.66
	Subsurface drip	I ₁	7	39.37	106.67	60.17	57.31	26.67
		I ₂	9	42.40	109.85	61.08	58.63	27.46
		I ₃	8	40.20	90.41	55.08	56.35	22.60
		I ₄	8	44.80	129.22	64.15	60.73	32.31
	LSD		ns	ns	ns	ns	ns	ns
	Drip		7	37.1	87.80	56.79	56.23 b	21.95 ns
	Subsurface drip		8	41.69	109.04	60.12	58.26 a	27.26
	LSD		ns	ns	ns	ns	1.258**	-
		I ₁	6 b	36.95	88.96	55.65	56.80	22.24 ns
		I ₂	8 a	39.50	96.35	58.81	58.83	24.09
		I ₃	7 a	39.03	92.45	56.23	56.34	23.11
		I ₄	8 a	42.10	115.93	63.13	57.01	28.98
		LSD	0.972*	ns	ns	ns	ns	ns

*: Significant at the p < 0.05,**: Significant at the p < 0.01, ns: Not significant a,b,c,...: LSD group

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Table 3. The onion quality parameters

Year	Irrigation method	Irrigation regimes	Dry matter content %	Soluble solids concn. %	pH	Protein content %	Total sugar content %	Invert sugar content %
2009	Drip	I ₁	14.65	9.33	5.60	1.39 bcd	9.61	2.29
		I ₂	12.65	11.57	5.58	1.80 a	9.11	1.95
		I ₃	14.16	10.70	5.45	1.83 a	8.66	2.64
		I ₄	15.04	11.64	5.60	1.18 d	9.67	2.30
	Subsurface drip	I ₁	14.72	8.77	5.58	1.50 bc	10.63	2.79
		I ₂	14.04	10.00	5.61	1.61 ab	10.42	2.12
		I ₃	14.33	10.50	5.52	1.22 cd	13.17	2.78
		I ₄	15.01	12.83	5.45	1.37 bcd	9.44	1.83
	LSD		ns	ns	ns	0.31 **	ns	ns
	Drip		14.13	10.81	5.56	1.55	9.26	2.30
	Subsurface drip		14.53	10.53	5.54	1.42	10.92	2.38
	LSD		ns	ns	ns	ns	ns	ns
		I ₁	14.69	9.05 b	5.59	1.45 ab	10.12	2.54
		I ₂	13.34	10.78 ab	5.60	1.70 a	9.77	2.03
		I ₃	14.25	10.60 b	5.49	1.53 ab	10.92	2.71
		I ₄	15.03	12.24 a	5.53	1.27 b	9.55	2.07
		LSD	ns	1.991 *	ns	0.63 **	ns	ns
2010	Drip	I ₁	12.65 bc	13.13	5.77	1.04	10.35	2.10 c
		I ₂	11.20 b	15.80	5.66	1.24	9.57	2.49 c
		I ₃	18.34 a	15.17	5.70	1.29	9.64	2.30 c
		I ₄	10.62 b	15.33	5.63	0.99	12.81	2.56 bc
	Subsurface drip	I ₁	16.67 ab	14.83	5.76	1.31	10.24	4.36 ab
		I ₂	17.27 ab	15.07	5.69	1.22	13.29	4.39 ab
		I ₃	18.70 a	15.00	5.69	1.30	9.28	1.83 c
		I ₄	14.41 bc	16.20	5.71	1.24	10.73	4.84 a
	LSD		3.03 *	ns	ns	ns	ns	1.86 *
	Drip		13.21 b	14.86	5.69	1.14	10.59	2.36 b
	Subsurface drip		16.76 a	15.28	5.71	1.27	10.89	3.85 a
	LSD		3.19 *	ns	ns	ns	ns	0.74 *
		I ₁	14.65 b	13.98	5.76	1.17	10.30	3.23 a
		I ₂	14.24 b	15.43	5.68	1.23	11.43	3.44 a
		I ₃	18.52 a	15.08	5.69	1.30	9.46	2.06 b
		I ₄	12.52 b	17.77	5.67	1.11	11.77	3.70 a
		LSD	2.44 **	ns	ns	ns	ns	1.07 *

*: Significant at the $p < 0.05$, **: Significant at the $p < 0.01$, ns: Not significant a,b,c,...: LSD groups

Conclusions

According to data obtained from this research, irrigation water quantities in the first year with 194.8 – 464.0 mm and in the second year with 145.5 – 341.3 mm was applied under Tekirdag located in the northwestern part of Turkey. The seasonal evapotranspiration was measured as 337.14 - 715.39 mm in 2009 and 388.79 – 579.26 mm in 2009. Also, seasonal

evapotranspiration increased with increasing irrigation water. The 6% less water consumption was averagely measured in subsurface drip irrigation method than drip irrigation method in both years.

The total marketable onion yield of under different irrigation methods, levels and cultivation year was highly variable. It ranged from 12.73 t ha⁻¹ to 25.99 t ha⁻¹ in 2009 and from 17.82 t ha⁻¹ to 32.31 t ha⁻¹ in 2010. These average marketable yield differences between years can be attributed the climatic conditions. Also, the total marketable onion yield increased with increasing irrigation water. The highest total marketable onion yields at rates of 20.13 – 27.26 t ha⁻¹ were obtained from subsurface drip irrigation in both years. Generally, the vegetative growth, yield and quality parameters were not significantly affected from irrigation method and irrigation levels.

As a result, the subsurface drip irrigation method is newly used in Thrace Region and Turkey conditions. Although, the surface drip irrigation has got high first installation cost and operation problems, it can be used for onion irrigation with high yield and less applied irrigation water.

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**MORPHOLOGICAL AND CHEMICAL PROPERTIES OF *Viola odorata* L.
DEPENDENCE OF SUBSTRATE AND FERTILIZATION**Erhatic R.^{1*}, Židovec V.², Herak Ćustić M.², Lončarić Z.³, Vukobratović M.¹¹ Križevci College of Agriculture, M. Demerca 1, HR-48260 Križevci, Croatia² Faculty of Agriculture, University of Zagreb, 10000 Zagreb, Croatia³ Faculty of Agriculture in Osijek, University J. J. Strossmayer, Osijek, Croatia

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Abstract

Sweet violet (*Viola odorata* L.) blooms in spring. It has delicate flowers that are often collected from natural habitats for their appealing fragrance. In ornamental horticulture it is used for cut flowers or planted in gardens. The whole plant is edible and curative, and there are numerous possibilities for its use for nutritional, pharmaceutical and cosmetic purposes. In order to achieve more successful breeding, special attention should be given to the substrate (better results are achieved in peat-based than in soil based substrate) and fertilization (nitrogen affects the foliage mass, and phosphorus, calcium and magnesium the quality). With the aim to obtain better information about selecting the most favorable substrate and the optimal dose of fertilizer, an investigation was conducted with five different types of commercial substrates, garden soil and soil from natural habitats and the foliar nutrition in four fertilization treatments (control, 0.1% NPK-Mg, 0.2% NPK-Mg and 0.2% NPK-Mg + 4% MgSO₄). Chemical analysis of the soil showed that the soil is medium with less organic matter, higher pH reaction and richer in potassium compared to a commercial substrate. Fertilization was carried out two times, and morphometric measurements of plant height, plant diameter, number of leaves and flowers, the colors of leaves and flowers were done before and after fertilization. The results of the measurements show that the plants grown in soil were larger in diameter and height, but had a smaller number of leaves. Also, we find that fertilization increased plant diameter by 6% and number of leaves by 13%, while plant height was not affected. Selecting the most acceptable media with optimal fertilization we can indirectly reduce the destruction of this nutritionally valuable and ornamental plant species in its natural habitats, and make it very interesting commercially.

Key words: growing media, morphometric properties, essential oils.

Introduction

Sweet violet is a well known medicinal, edible and ornamental plant which blooms with flowers of attractive scent early in the spring, in the conditions of continental climate (Erhatic et al., 2010). It grows wild in nature, in places exposed to sun, alongside hedges, river banks, on the edges of deciduous forests and in forest glades (Willfort, 2002; Černicki, 2006). It is wide-spread all across Croatia, along with the mentioned species; Domac (2002) lists further 19 species of the same genus.

The Mediterranean is considered to be the sweet violet's original habitat (Köhlein, 1999), and nowadays it ranges from the North of Europe to South Africa, Tierra del Fuego and Australia. Early studies indicate that the soil for growing violets needs to be fresh, fertile, well cultivated and deeply treated during autumn or winter and fertilized with a sufficient amount of composted manure (Erhatic at al., 2010). According to more recent data, the violet grows mostly on alkaline soils (Bagci et al., 2008), but it can also be found on soils ranging from acidic to alkaline. Soils from natural habitats contain a significant amount of organic matter (6-11%). Organic matter has a positive effect on plant growth because it contributes to the improvement of the physical, chemical and biological soil properties, mineralization of nutrients and therefore, on the plant growth itself (Makineci et al., 2007). Violets are usually found on soils with less phosphorus and larger potassium content (Makineci et al., 2007, Bagci et al., 2008). When growing plants in substrates, peat and compost based substrates gave the best results: the greatest height of plants, the greatest mass and leaf surface, the longest root. This was found for numerous plant species like: lettuce, cucumbers, peppers, strawberries and forest plants. Substrates based on soil, however, gave the weakest results (Erhatic at al., 2010). The aim of this study was to investigate the influence of the substrate, fertilization and their interaction on some morphometric characteristics of sweet violet: plant height and diameter, number and colour of leaves and flowers and some chemical characteristics, such as the amount of essential oils in the leaves of sweet violet.

Materials and methods

In June 2009 plants have been propagated by dividing and 320 plants per soil or substrate type have been planted in two soil types (forest and garden soil) and five different substrates produced by mixing different basic raw materials (Table 1). Before planting, the chemical composition of the soil was tested in the laboratory of the Križevci College of Agriculture (Page, 1982), while the chemical composition of the substrate was read out from the declaration. During the research, violets were fertilized in four levels of fertilization:

1. variant without adding fertilizer (C),
2. „KRISTALON“ - 0,1% NPK-Mg lower level (NPK-L),
3. „KRISTALON“ - 0.2% NPK-Mg higher level (NPK-H),
4. „KRISTALON“ - 0.2% NPK-Mg higher level with the addition of 4% MgSO₄

(NPK-Mg)

A two factorial experiment was set in a randomized block design with four replications, and each combination was represented with 20 plants. During winter of 2010 and spring of 2011, the following morphometric properties of sweet violet plants were measured:

1. plant height (cm)
2. diameter of plants (cm)
3. number of leaves (n)
4. number of flowers (n)
5. colour of leaves and flowers

Leaf and flower colour was determined by the RHS colour chart. To determine the overall concentration of essential oils in the leaves, the official standard Clevenger distillation method was used (Official Methods of Analysis, 1998 Volatile oil in spices, AOAC International). Fertilizer was applied only twice (June 9th and June 25th, 2011). Due to low temperatures and bad weather conditions it was not possible to fertilize the plants earlier.

The effects of substrate, fertilization and their interactions were tested with a two-way variance analysis.

Results and discussion

Substrate analysis

The chemical properties of substrates and soils are shown in Table 1. The reaction of the substrates ranges from pH 5.5 to 5.8, and the pH of soils from 7.1 to 7.7. According to the measured pH reaction, the substrates are defined as mildly acidic, while the soils are neutral. Although this reaction is suitable for the cultivation of most plant species, it seems that the sweet violets prefer a higher pH so the plants grown in forest soil had the greatest diameter, maximum height and greatest number of leaves.

The measured electrical conductivity (EC) is in the range from 0.35 to 0.90 dS m⁻¹. Low EC can mean a low fertility, because of the low nutrient content, but on the other hand, a high salt concentration may indicate potential phytotoxicity. Since the desirable value of EC for media that are intended for breeding of plants, ranges from 0.5 to 1.8 dS m⁻¹ (Kuo, 2004), we conclude that the substrates in the study are suitable for growing violets.

The substrates and soils are rich in organic matter (Table 1). The content of basic macroelements in substrates varies in a very wide range (N 100-280 mg l⁻¹; P₂O₅ 130-240 mg l⁻¹ and K₂O 180-360 mg l⁻¹), and the soil is well supplied with nitrogen, poorly supplied with phosphorus and medium to richly supplied with potassium.

Table 1. Chemical properties substrates and soils

Substrates	pH _{H2O} (1:10)	EC dSm ⁻¹ (1:5)	Organic matter (%)	N mg l ⁻¹	P ₂ O ₅ mg l ⁻¹	K ₂ O mg l ⁻¹
Substrate 1	5.8	0.35	80	150	170	190
Substrate 2	5.8	0.60	90	280	200	360
Substrate 3	5.6	0.90	85	100	130	180
Substrate 4	5.5	0.45	80	210	150	270
Substrate 5	5.8	0.50	90	210	240	280
Soils	pH _{H2O}	pH _{KCl}	Humus (%)	N	AL-P ₂ O ₅	AL-K ₂ O
				mg kg ⁻¹		
Forest soil (F _s)	7.7	6.8	4.93	3.8	3.82	15.54
Garden soil (G _s)	7.1	6.5	4.57	3.5	3.47	68.95

Plant diameter and height

Measurement results (Table 2) show a statistically important difference in plant diameter, depending on the type of substrate and the date of measuring ($P < 0.0001$). The average maximum diameter of the plants independent of the measurement date was found on the forest soil (11. cm). The smallest plants were on the substrates S₂ and S₄ (8 and 9 cm). According to measuring dates, the biggest diameter was measured at the beginning (13 cm). In winter it was declining, and rising up in the spring until the end of measuring. By following the development of sweet violets, according to the substrates and dates of measurement, the diameter in all the studied substrates decreased in the period from November 2010 to April 2011. The most significant decline was noted on the substrate S₁ (109%), while the smallest decline was on forest soil (49%) and garden soil (63%).

SECTION 5. VEGETABLE, FLOWER AND DECORATIVE PLANTS PRODUCTION

Table 2. The diameter of the plants during the growing season of sweet violets per substrates before fertilization (cm)

Substrate/date	F _s	G _s	S ₁	S ₂	S ₃	S ₄	S ₅	Average
14/10/2010	15	15	14	12	13	11	14	13 A
9/11/2010	13	11	12	10	12	11	13	12 B
15/12/2010	11	10	8	8	9	9	14	10 C
19/01/2011	10	9	7	7	8	8	11	9 DE
21/02/2011	10	9	7	7	8	7	9	8 FG
17/03/2011	10	9	7	6	8	7	8	8 G
15/04/2011	11	9	7	7	8	7	10	8 FE
12/05/2011	12	9	10	9	8	9	8	9 D
30/05/2011	12	10	11	8	11	9	10	10 C
Average	11 A	10 B	9 C	8 D	9 C	9 D	11 B	10

Statistically significant difference in plant diameter was registered amongst substrates and fertilization treatments (Table 3). Plants grown on forest soil and garden soil had the biggest diameter, whilst those grown on commercial substrates had smaller diameters. Depending on fertilization, the best results were achieved using NPK-Mg treatment.

Table 3. Diameter of sweet violet plants after fertilization performed on substrates (cm)

Substrate/fertilizer	F _s	G _s	S ₁	S ₂	S ₃	S ₄	S ₅	Average
8Control	12 ns	13 ns	13 ns	11 ns	12 ns	11 ns	13 ns	12 B
NPK - L	14 ns	13 ns	13 ns	10 ns	12 ns	11 ns	14 ns	12 AB
NPK - H	12 ns	14 ns	11 ns	13 ns	12 ns	11 ns	11 ns	12AB
NPK -Mg	15 ns	12 ns	13 ns	11 ns	13 ns	11 ns	12 ns	12 A
Average	13 A	13 A	12 AB	11 B	12 AB	11 B	12 AB	12

There is also statistically important difference between plant heights depending on substrates and measuring dates (Table 4). The highest plants were measured on forest soil (6 cm), and the shortest plants on garden soil (4 cm). According to measuring dates, the highest plants were measured at the beginning of measurement (6 cm). In winter the height was declining, but it was rising up in the spring until the end of measuring 6 cm).

Table 4. Height of plants during the growing season of sweet violets per substrates before fertilization (cm)

Substrate/date	F _s	G _s	S ₁	S ₂	S ₃	S ₄	S ₅	Average
14/10/2010	7	6	6	6	5	6	5	6 A
9/11/2010	5	5	5	5	4	6	5	5 C
15/12/2010	5	4	3	3	3	3	4	3 E
19/01/2011	5	3	3	3	3	3	3	3 F
21/02/2011	5	3	3	3	3	3	3	3 F
17/03/2011	5	3	3	3	4	3	3	4 E
15/04/2011	6	3	4	4	4	4	5	4 D
12/05/2011	6	4	6	5	6	5	6	5 B
30/05/2011	7	4	6	6	6	5	6	6 A
Average	6 A	4 D	4 C	4 BC	4. CD	4 BC	5 B	4

After the fertilization, the highest plants were developed on forest soil (9 cm), and they showed a slight difference compared to the plants grown on the substrate S₅ (8 cm). Significantly lower plants were developed on the substrates S₃ and S₄, and they were

comparable to the plants grown on the garden soil, and substrates S_1 and S_2 . Different fertilization had no significant impact on plant height (Table 5).

Table 5. Plant height of sweet violets upon completion of fertilization per substrates (cm)

Substrate/ fertilizer	F_s	G_s	S_1	S_2	S_3	S_4	S_5	Average
Control	8 ns	7 AB	7 ns	7 ns	6 ns	7 ns	7 ns	7 ns
NPK - L	10 ns	7 B	8 ns	9 ns	7 ns	7 ns	9 ns	7 ns
NPK - H	8 ns	8 A	9 ns	7 ns	7 ns	7 ns	9 ns	8 ns
NPK - Mg	10 ns	7 B	8 ns	7 ns	7 ns	7 ns	8 ns	7 ns
Average	9 A	7 BC	8 BC	8 BC	7 C	7 C	8 AB	7

The results are in accordance with the values specified by Eckstein et al. (2006), Židovec (2008), Erhatic et al. (2010) in their research of the same morphological parameters in violet varieties depending on habitat and type of substrate used. Dubsy and Sramek (2009) and Vukobratović (2008) state that better results of growth parameter (height and mass of plant) were achieved on peat-based substrates and compost rather than soil-based ones. The impact of foliar fertilization on flower height was researched by Čustić and Poljak (1994) and it shows better results in the application of fertilizer.

Number and colour of leaves and flowers

Plants with the largest number of leaves ($P < 0.0001$), independent of the measurement date, were found on the substrate S_5 (11) and they were comparable to the ones grown on the forest soil (11). Plants with a significantly lower number of leaves were found on other substrates (Table 6).

Table 6. Number of leaves during the growing season of sweet violets before fertilization per substrates (cm)

Substrate/date	F_s	G_s	S_1	S_2	S_3	S_4	S_5	Average
14/10/2010	13	17	13	10	13	9	18	13 A
9/11/2010	13	15	13	10	13	10	16	13 A
15/12/2010	11	11	10	8	10	8	15	10 B
19/01/2011	10	10	8	7	8	7	12	9 D
21/02/2011	10	8	8	6	7	6	10	8 E
17/03/2011	10	8	8	6	6	6	6	7 F
15/04/2011	10	8	8	6	6	6	8	7 EF
12/05/2011	10	9	9	7	8	7	8	8 ED
30/05/2011	11	10	10	8	9	7	10	9 C
Average	11 AB	11 B	10 C	8 D	9 C	7 D	11 A	9

Plants with the largest number of leaves, independent of the substrate, were recorded in October and November 2010 (13 and 13), while the number of leaves was smaller in other dates of research. At the end of the study, upon completion of fertilization, the highest number of leaves (Table 7), independent of the substrates, was recorded with the fertilizer NPK-Mg (11). It was comparable to the fertilizer NPK-H (11), but higher than the control sample (10) and the fertilizer NPK-L (10). The highest number of leaves, independent of fertilization treatments, was recorded with the garden soil (12), and it is comparable with the number of leaves on the forest soil (12) and the substrate S_5 (11). Plants with an absolute minimum number of leaves were developed on the substrate S_1 (7) and maximum

on forest soil (16). The study shows that the number of leaves depends on fertilization and quality of substrate and is in line with Ayodele (2002), Židovec and Vršek (2007) and Erhatic et al. (2010). The most common leaf colours before and after fertilization are 137A and 138A green, which is in line with the research of Židovec (2009).

Table 7. Leaf number of sweet violet plants after completion of fertilization per substrates (cm)

Substrate/ fertilizer	F _s	G _s	S ₁	S ₂	S ₃	S ₄	S ₅	Average
Control	9 B	14 ns	9 ns	9 B	10 AB	9 ns	10 ns	10 B
NPK - L	11 B	12 ns	9 ns	11 AB	9 B	8 ns	11 ns	10 B
NPK - H	12 AB	13 ns	8 ns	13 A	11 AB	11 ns	12 ns	11AB
NPK -Mg	16 A	11 ns	7 ns	11 AB	13 A	10 ns	13 ns	11 A
Average	12 AB	12 A	8 D	11 ABC	11 BC	9 CD	11AB	11

The number of flowers was only investigated before fertilization as in the period after fertilization there were no blooming plants. The plants with the average maximum number of flowers were found in May 2011 on the substrate S₅, and the most common flower colour was 86 A purple, which coincides with the research of Židovec (2009).

The amount of essential oils in sweet violet leaves

There is a statistically significant difference in the amount of essential oil independently of the substrates (Table 8). The largest amount of essential oil in the sweet violet leaves was determined with NPK–H (0.019%) and NPK-Mg (0.015%). A significantly smaller amount of essential oil was recorded in the control sample (0.005%) and in plants treated with NPK-L (0.006%). Independently of the fertilization, the values vary from 0.009% to 0.014% essential oil in the sweet violet leaves. The values of the amount of essential oil in the sweet violet leaves are somewhat lower than the values recorded by Flamini et al. (2002), Erhatic et al. (2011).

Table 8. Essential oils in the leaf of sweet violet per substrates and fertilizers (%)

Substrate/ fertilizer	F _s	G _s	S ₁	S ₂	S ₃	S ₄	S ₅	Average
Control	0.004 B	0.013 ns	0.002 B	0.002 C	0.005 B	0.004 C	0.004 ns	0.005 B
NPK - L	0.005 B	0.010 ns	0.005 B	0.004 BC	0.004 B	0.006 BC	0.006 ns	0.006 B
NPK - H	0.014 A	0.018 ns	0.018 A	0.014 AB	0.020 A	0.015 A	0.033 ns	0.019 A
NPK -Mg	0.012 A	0.015 ns	0.015 A	0.018 A	0.015 AB	0.013 AB	0.015 ns	0.015 A
Average	0.009 ns	0.014 ns	0.010 ns	0.009 ns	0.012 ns	0.009 ns	0.014 ns	0.011

Following the impact of the substrate on the growth of sweet violet (Table 2, 4, 6), we can conclude that highest plants, with the largest diameter and with the greatest number of leaves are grown on forest soil. This is contrary to our expectations that better results will be achieved on substrates with more organic matter and lower pH reaction. With respect to the other results of measurements, we conclude that the sweet violet requires substrate rich

in nutrients in well-balanced relationship. Supply of nitrogen has greater influence on the growth (Fs, S5, S1 and S3), while a good balance of nutrients influences the diameter and number of leaves (Fs, S5, S2 and S4). If the research was extended to the determination of the physical properties of soil and substrate, the conclusions would be made with greater certainty. One of the reasons why the forest soil gave the best results is its better water retention and less porosity. Research should certainly be repeated and possibly expanded to the cultivation in protected areas.

Conclusions

The observation of the impact of the substrate and fertilisation on the growth of the sweet violet has only partially confirmed the hypothesis of the vegetative growth of the sweet violet being greater on the substrate with more organic compounds. Only the height and the diameter of sweet violet plants were greater on the substrate with less organic compound (garden soil and forest soil), while the leaf number was greater on the substrate with more organic compound. The same situation repeated with the pH of the tested substrates. The hypothesis of the vegetative growth of sweet violet plants being greater on the substrate with a lower pH value was also only partially confirmed. Only the height and the diameter of sweet violet plants were greater on the substrate with a greater pH reaction (garden soil and forest soil), while the number of the leaves was greater on the substrate with a lower pH reaction. The maximum amount of essential oils in the leaf of sweet violet was determined with the plants developed on the garden soil, and it was identical to the amounts determined on the substrate S₅ (0,014%). Based on the results of the conducted research, it may be concluded that the most favorable medium for the cultivation of sweet violet is the forest soil, along with the fertilizer NK – higher level and NPK – higher level with the addition of Mg.

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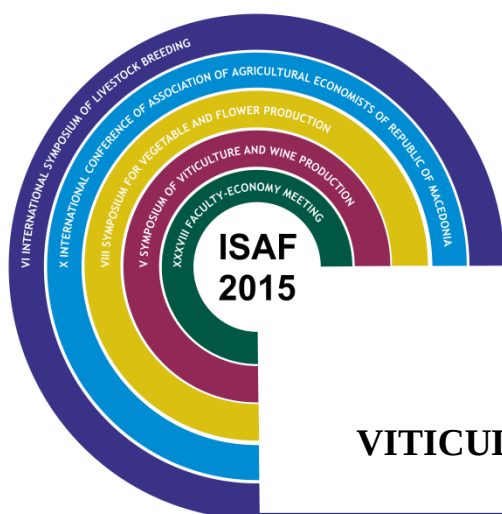
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Abstract

Congeners (lat. "born together") are components which are generated in many alcoholic beverages as byproduct during alcoholic fermentation. These are series compounds that lead to formation of a complex aroma and flavor of wines and the greatest impact they express in hard alcoholic drinks. Acetaldehyde, n-propanol, isobutyl alcohol, isoamyl alcohol, and amyl alcohol produced with smaller amounts of other higher alcohols, and various esters, also called fusel oils are components which are present in wine. The pharmacologically active ingredients that are responsible for toxicity and hangover symptoms on humans are not alcohol itself or aliphatic alcohols, but their metabolites, which include also their aldehydes. For that reason in this paper were determined beside ethanol, several compounds (except aldehydes) that fall into so-called congeners in seven types of wines using direct GC-FID analysis of wines or their distillates obtained by classic laboratory distillation. This method was easy way for determination of some congeners in wines. A comparison of results for the presence of the corresponding component in wines and distillates showed that red wines were generally richer in congeners against the whites. A lower concentration of ethanol, methanol, ethyl acetate and n-propanol were obtained in white wines. Iso-amyl alcohol had a higher concentration in white wines and isobutanol had different concentration in examined wines.

Key words: congeners, direct GC-FID analysis, wines

Introduction

Alcoholic beverages are produced during fermentation of sugars by yeast for many years ago. As yeasts fermented the sugars from must and other sugars rich source they create ethanol and carbon dioxide, but they also produce a range of other compounds which are responsible for their specific taste and smell and some of them can be designated as congeners. Most of the flavor compounds of alcoholic beverages are present in extremely low concentrations (congener alcohols < 5 000 mg/l, aldehydes, carbon acids <2 mg/l and higher esters, <0.001mg/l), but some can be found in sufficient quantities to allow

detection of them in the blood of consumers. These are the congener alcohols: methanol, propan-1-ol, butan-1-ol, butan-2-ol, isobutanol, 2-methylbutan-1-ol and 3-methylbutan-1-ol. Methanol is a breakdown product of the vegetable or grape pectin. The higher aliphatic alcohols are byproducts of the fermentation process and have their origin in amino acids whose amino groups are needed for the nitrogen supply of yeast. Higher alcohols as congeners are produced either from sugars and from amino acids also by Erlichmechanismas the higher aliphatic alcohols were originally extracted from fusel oils (i.e. the third phase of the distillation procedure) they are also called fusel alcohols (Ribereau-Gayonet *al.*, 2006). There are characteristic differences in the congener content of fermented alcoholic beverages uch as beer, wine, rice wine etc. They contain only small amounts of methanol, propan-1-ol, isobutanolandisoamyl alcohols. Distilled spirits contain the same congener alcohols as fermented beverages, but in different, sometimes very high quantities. Other spirits, such as fruit brandies are characterized by extremely high concentrations of methanol or contain butan-2-ol as a specific component (Kostiket *al.*, 2014a,b). Acetaldehyde, C3 alcohol (n-propyl alcohol), C4 alcohol (isobutyl alcohol) and C5 alcohols (isoamyl alcohol, active amyl alcohol) with smaller amounts of other higher alcohols, and various esters, also called as fusel oils are components which are presents in wine (Ribereau-Gayon *et al.*, 2006). Some of them had been investigated about their toxicity and hangover symptoms or effects of humans.The hypothesis thatsom congeners have acute or post-acute effects on human bodywas confirmed. The pharmacologically active ingredients thatare responsible for this phenomenon is not alcohol itself or aliphatic alcohols, but their metabolites, which include also their aldehydes.Also, modern analytical methods allow their detection in the blood of drunken drivers and alcohol congener analysis are examined in forensic application for determining the source of alcohol or alcoholic beverages(Swiftand Davidson, 1998; Rohsenow and Howland, 2010; Jung *et al.*,2010, 2012, 2013). For that reason, in this paper were determined beside ethanol, several compounds that fall into so-called congeners in different types of wines using direct GC analysis of wines and also direct GC analysis of their distillates obtained by classic laboratory distillation of examined wines.

Experimental part

Seven different typesof wines (from local suppliers were used for analysis of few congeners. Three were white wines (Ab, Sm, Tr) with declared alcohol content 11,5%, 10,5% and 12%, three were red wines (Ac, Ka, Tg) with alcoholic contents 11,5%,11% and 12,5%, one was rose wine (R)with 12,5% alcohol.For determination of congeners in wine the same samples were distilled using laboratory standard equipment. All investigated wines or their distillates were analysed directlyusingGas chromatography (GCmodelVarian CP 3800)equipped with a split/splitless injector and a flame ionization detector (FID). For separations of components capillarycolumncoated with CP-Wax 52 CB(30 m x 0.32 mm id. x 0.25 µm film thickness) was used. The injection port wasmaintained at 220°C and temperature of detector (FID) at 250°C, manual injector type 1709 and gases: nitrogen (30 ml/min), air and hydrogen (300 ml/min).The oven temperature was held at: 40°C (1 min), increasing to 100°C with rate 4°C/min (held 2 min) and finally increasing temperature with rate 10°C/min to 200°C (held 2 min). The volume of injected samples was 2 µl and total time of analysis was 30 min.

Qualitative and quantitative identification of the analytes was based on retention time in the column and appearance of the peak signal as compared with the standard solution with known substances and with using internal standard. The standard chemicals: ethanol, methanol, isoamyl alcohol, isobutanol, n-propanol, ethylacetate, isoamyl acetate, n-amyl alcohol, n-butanol were 90-99% p.a, and acetonitril as internal standard (IS) was used for obtained of calibration curve (concentration versus area ratio P_i/P_{std}). The concentration of each i-analyte was determined with respect to the IS from the relative response factors (RRF), which were obtained during calibration under the same chromatographic conditions as those of the sample analysis. Identification of components was performed by comparing retention indices with those of pure standard compounds.

Results and discussion

Analysis of some congeners in wine was conducted because some of them have negative influence in human body. After drinking, the alcohols were rapidly absorbed through the gastrointestinal mucosa and distributed throughout the body and very little percentage was excreted in urine. Methanol, propan-1-ol, butan-2-ol and ethanol are only soluble in body water. Isobutanol and the isoamyl alcohols have a much greater distribution volume because they are also soluble in lipids. The fusel alcohols (not methanol) were partly or completely conjugated with glucuronic acid, this effect being more significant the longer the carbon chain of the alcohol and accompanied by an increase in the water solubility. However, only 5-10% of the consumed congener alcohols were excreted in free or conjugated form in the urine. This means that their elimination is mainly due to metabolism. The accumulation of methanol by exogenous supply lead to a production of the pharmacologically highly active metabolite formaldehyde during the post alcoholic phase, when the inhibition of alcohol dehydrogenase by ethanol has ceased. This would explain the heavier hangover symptoms after the consumption of congener-rich beverages, which usually also contain higher amounts of methanol.

The first step of congeners analysis include determination of their retention time (mean value), such as their standard deviations and relative retention time RRT (Table 1). After carrying the analysis of the standards alcoholic solutions of seven components and determined area ratio of peaks of analysing components and internal standard was calculated concentration of congener from obtained calibration curve. The linear regression equations with corresponding correlation coefficient (R^2) are shown in Table 2.

Table 1. Mean value of retention time (RT) and relative retention time (RRT), and their standard deviation and percentage of standard deviation for all injected standard solutions of nine analysed components

Components	Mean value of retention time(RT _{mean})	Standard deviation of RT _{mean}	% standard deviation of RT _{mean}	Mean value of RRT	Standard deviation of RRT	% stand deviation of RRT
Acetonitril (IS)	5.209	0.015	0.284	1.000	0.000	0.000
Ethanol	4.121	0.035	0.846	0.791	0.005	0.620
Ethyl acetate	3.576	0.011	0.306	0.687	0.001	0.195
Isoamyl acetate	8.958	0.117	1.304	1.720	0.020	1.192
Isoamyl alcohol	10.782	0.140	1.295	2.070	0.026	1.250
Iso-butanol	7.230	0.047	0.652	1.388	0.007	0.479
Methanol	3.690	0.014	0.383	0.708	0.003	0.437
n-amyl alcohol	12.019	0.117	0.974	2.307	0.022	0.946
n-butanol	7.609	0.077	1.005	1.461	0.013	0.896
n-propanol	5.808	0.035	0.606	1.115	0.005	0.410

Table 2. Calibration equations obtained for calculation of concentration for nine components (y, mg/l) against P_i/P_{std}ratio(x) aafter direct GC-FID analysis

Components	Equation	R ²
Ethanol	y = 0.8687x - 1.092	0.9906
Ethyl acetate	y = 0.5451x + 0.0008	0.9982
Iso amyl acetate	y = 0.3455x - 0.0009	0.9999
Iso-amyl alcohol	y = 0.2781x + 0.0011	0.9748
Iso-butanol	y = 0.315x - 0.0024	0.9985
Methanol	y = 0.5872x + 0.0037	0.9938
n-Amyl alcohol	y = 0.2956x - 0.0001	0.9996
n-Butanol	y = 0.4518x - 0.0027	0.9806
n-Propanol	y = 0.3622x + 0.0024	0.9811

Linearity is an important part of the analyzing congener, but good correlation coefficients $R^2 > 0.9950$ which is very important for all components was not obtained for all cases. Direct analysis of components with GC-FID is very simple methods but for some components with smaller concentrations present in wine this method gave higher error or some of them were not detected. Correct concentration of each component (C_i) in the examined wines was determined by equation (1) and relative percentage of standard deviation of RRF with equations 2 and 3:

$$C_i = (P_i/P_{std}) \cdot C_{std}/RRF_i \cdot 1/V \quad (1)$$

$$SD_{RRF} = \sqrt{(\sum (RRF_i - RRF_{mean})^2 / N - 1)} \quad (2)$$

$$\%RSD = SD_{RRF} / RRF_{mean} \times 100 \quad (3)$$

Where: RRF -relative responsible factor calculated from equation $RRF = (P_i/C_i) / (P_{std}/C_{std})$

P_i/P_{std} - ratio of area of analysed i-component (P_i) and area of internal standard (P_{std})

V-volume of injected sample in ml

N- number of injected sample

C_{std} - concentration of internal standard

SD_{RRF} - standard deviation of RRF

%RSD - relative percentage of standard deviation

For each analysed congeners from injected standard solutions were obtained higher value than statistic data that % RSD must be $\pm 20\%$ especially for component n-butanol and isoamyl alcohol. It suggested that it will be useful for using other internal standard or using smaller concentration of standard solutions of congeners to obtain value with smaller relative standard deviation and to obtained real concentration of congeners in wine. Independent of that, all obtained results for determined concentrations of congeners with direct analysis of wines or their distillates are presented in Figure 1. After running the wine samples, the end of the analysis showed no peaks for congeners iso-amyl acetate, n-amyl alcohol and n-butanol in wines or their distillates. Their likely low concentration in wines cannot be determined according to this applied GC method.

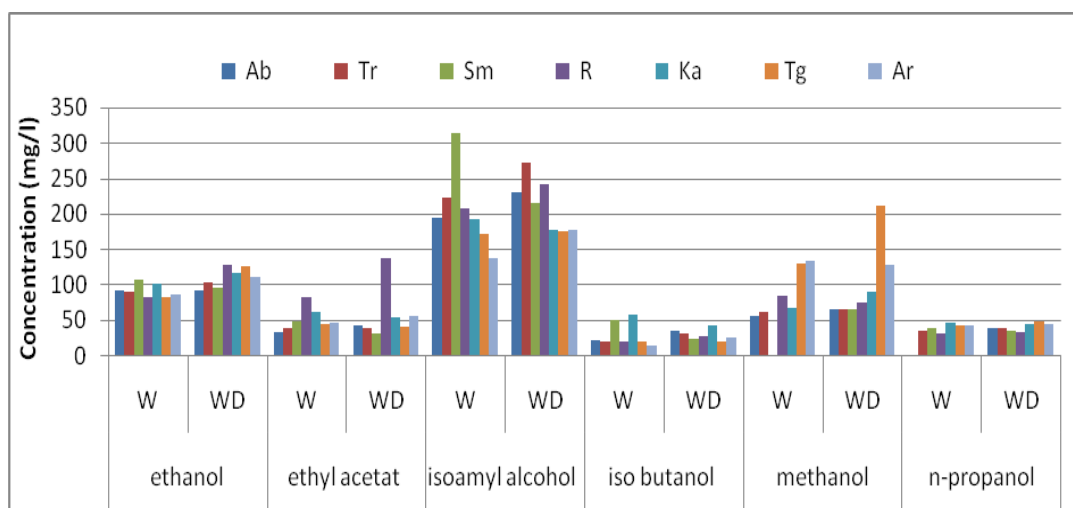


Figure 1. Determined concentration of components in nine different wines (W) and in their wine distillates (WD)

In all wines determined concentration of ethanol with direct analysis of wines or their direct analysis after conducted laboratory distillation were obtained some difference. Having in mind that ethanol which is main component present in higher amount in wines than other components and although it had linear equation with $R^2 = 0.9906$, determined concentrations was relative similar or closely to declared contents of ethanol. Results for concentrations determined after distillation of wine and their injection in GC, was little higher or smaller in some wines then that obtained with direct analysis of wines and that calculated difference are maybe due to the higher relative standard deviation % RSD 32.67. For correct or really determination of concentration of ethanol or other congeners in wines is very important to know that distillation or laboratory distillation is not only capable of increasing the alcohol by volume percentage (ABV) but can also alter to the relative congener concentrations produced which is shown in Table 3. However, when congener concentrations are compared to the concentration of ethanol (i.e., congener/ABV), the relative difference in congener concentration between fermented and distilled beverages is reduced, citrated Rodda *et al.* (2013).

Table 3. Ratio of determined concentration of congeners in wine (W) versus distilled wine (WD)

Components→ wines↓	ethanol	ethyl acetate	iso amyl alcohol	iso-butanol	methanol	n-propanol
A _b	1.002	0.788	0.846	0.635	0.836	0
T _r	0.879	1.003	0.813	0.641	0.933	0.886
S _m	1.132	1.588	1.461	2.199	0	1.1045
R	0.649	0.595	0.859	0.741	1.137	0.908
Kr	0.877	1.150	1.087	1.388	0.748	1.020
Tg	0.663	1.101	0.981	1.003	0.611	0.891
A _r	0.782	0.831	0.783	0.581	1.045	0.945

Ethyl acetate can be formed in wine through the action of yeast or separately via esterification (reaction of ethanol and acetic acid). Yeasts, present throughout fermentation, normally create the majority of ethyl acetate within a wine. The final concentration created in this way is dependent upon the species of yeast and the initial constituents of the wine or wine contamination with *Acetobacter spp.* And other aerobic bacteria. Ethyl acetate is the most abundant ester found within wine with threshold value about 160 mg/l. Below this value, while it may not be identifiable, it may spoil the bouquet with an unpleasant, pungent tang. It is, however, possible that at very low doses (50–80 mg/l) ethyl acetate contributes to a wine's olfactory complexity and thus has a positive impact on quality (Ribereau-Gayon *et al.*, 2006).

Determined concentration of ethyl acetate in directly analysed white wines Ab, Tr, and Sm were consequentially 32.92, 38.10 and 48.45 mg/L, but in their distillate determined concentration was different with respectively accounted W/WD ratio (Table 3). In red wines Ka, Tg and Ar concentration of ethyl acetate were 45.13, 44.08 and 61.06 mg/l, but in rose wine it was higher 81.48 mg /l or 136.88 mg/l in their distillate. Appropriate conducted distillation of wines gives different calculated concentrations of congeners in all wines. It suggested that during distillation it has been concentrated in some distillate of wines or volatile in some of them.

The concentrations of ethyl acetates in all our white wines was generally below 50 mg/l, and similar with literature data. Commercial *S. cerevisiae* strains produced less ethyl acetate than epiphyte microorganisms and can produce various concentrations of acetates of higher alcohols and fatty acids in different wines (Herjavec *et al.*, 2003), but also yeast assimilable nitrogen content of grape musts can cause some effect on producing esters by different yeasts strains (Carrauet *et al.*, 2008). In comparison to white wine from other countries, lower concentration ranges of ethyl acetate has been obtained for Chardonnay wines (n.d.- 7.90 mg/l). In Colombar and Chenin blanc ethyl acetate were 39.30–48.30 mg/l (similar levels like our wines) and in some Portuguese wines levels of ethyl acetate varied from 27 - 65 mg/l (Louwet *et al.*, 2010). Only rose wine had higher concentration which was 81.48 mg/l.

The level of isoamyl alcohol (3-methyl-1-butanol) was higher in five distillates of wines than their level in direct analyzing same sample of wines (Figure 1). Quantitatively, isoamyl alcohol generally accounts for more than 50% of all fusel alcohol fractions in which are included 1-propanol, 2-methyl-1-propanol (isobutyl alcohol), 2-methyl-1-butanol and isoamyl alcohol (Muller *et al.*, 1993). We found in the largest quantity in wine, isoamyl alcohol which concentration reaches 272.8 mg/l in white wine Tr. Isoamyl alcohol is the main aliphatic fusel alcohol synthesized by yeast during fermentation and depending

on the nature of the wine or beverage, it comprises 40-70% of the total fusel alcohol fraction (Lambrechts and Pretorius, 2000). Viticultural conditions and the use of different yeasts contribute to considerable variations in the quantity of higher alcohols which are also important precursors for ester formation and the esters of higher alcohols are associated with pleasant wine aromas. Isoamyl alcohol was determined by Herjavec *et al.* (2003), in Chardonnay wines at various concentrations (from 166 -278 mg/l) which were dependent of yeast strains. Although, the major four higher alcohols found in wines in order of amounts produced are isoamyl alcohol, active amyl alcohol, isobutyl alcohol and n-propyl alcohol with numerous other constituents in lower concentrations (Margalit, 1997), isobutanol and n-propanol as congeners were detected in small amounts in our wines and their distilled samples, up to 57.17 mg/l isobutanol and 45.02 mg/l n-propanol in red wine Ka. Isobutanol is the final product of the catabolism of valine in *S. cerevisiae* and it tends to increase in content during wine aging (Dickinson *et al.*, 1998).

Methanol which has a determination coefficient of $R^2=0.9938$ in our results, meaning that 99.38% of the total variation of concentration can be explained by a linear relationship between C and Pi/Psdt, while the remaining percentage of the variation continues to be unexplained. Methanol levels were ranged from 54.89 in white wine Ab to 133.4 mg/l in red wine Ar. Our results compared with Turkish wines (Cabaroğlu, 2005) obtained from different representative viticultural regions, confirmed that red wines have higher concentration of methanol due to maceration or pectolytic enzyme treatment used in red wine production. Their methanol levels are ranged from 30.5 to 121.4 mg/l in white wines, from 62.5 to 84.6 mg/l in rosé, and from 61.0 to 207.0 mg/l in red wines.

Distillation separates ethanol and volatile congeners based on their boiling points, with congeners of similar or lower boiling points. This is concentration effect which was demonstrated in our wines especially for methanol. In all samples of distilled wines after injected into GC were determined higher methanol levels, up to 211.2 mg/l in red wines Tg. All the results show that methanol levels are under the maximum acceptable limits of International Office of Vine and Wine (OIV) and do not represent a risk to consumer health. Wines and other fermented beverages generally containing lower concentrations of total congeners per volume when compared with distilled beverages (Rodda *et al.*, 2013).

At the end, it is very important to say that when Romano *et al.* (2003) analyzing fifty-two *S. cerevisiae* strains, isolated from different grape varieties, shows the behaviour of the strains for the production of n-propanol, isobutanol and isoamyl alcohol, acetaldehyde, ethyl acetate and acetic acid. The strains exhibited a low variability in the levels of n-propanol, acetaldehyde and ethyl acetate, whereas the other compounds were formed with significant strain variability. These results lead to consider the production level of these compounds as an individual strain characteristic and underline the importance of characterizing strains for industrial purpose.

Conclusion

A comparison of results for the presence of the corresponding component in wines and distillates showed that red wines are generally richer in congeners against the whites. Indeed lower concentrations of ethanol, methanol, ethyl acetate, and n-propanol was obtained in white wines, while iso-amyl alcohol was a higher concentration, compared to red wine. The concentration of isobutanol has higher variation in different white and red

examined wines. Although large variations in congeners was obtained in our results and in different countries and different grape varieties, the higher concentrations of methanol or aldehydes have much higher effects on hangover symptoms or toxic effects in people which drinking red wines or higher legal alcoholic beverages. The main group of compounds that form the “fermentation bouquet” are organic acids, higher alcohols and esters and to a lesser extent acetaldehyde, but when are present congeners in excess concentrations, these compounds may also be regarded as undesirable. For that reason easy and simple methods are always needed for analyzing congeners in wine or alcoholic beverages.

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UDC: 634.862/.863-152.61(497.7/4)
Original Scientific Paper

YIELD PLANNING AND QUALITY OF PALIERI TABLE GRAPE VARIETY IN TIKVES WINE REGION

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Abstract

The research has been conducted on the Palieri vine cultivar in Tikves wine region, purposed for production and consummation of fresh grape in Tikvesh wine region. To improve the quality of the table grape it is needed application of suitable ampelotechnical measures such as: yield planing with mature pruning, leaving specific numbers of grape clusters per vine, pincage of grape cluster, partial defoliation, use of bioregulators and application of modern systems of packing. Research was conducted in a direction of yield planning and quality of the grape with leaving 19.24 and 29 clusters per grape on both varieties and leaving of all clusters per grape taken as a standard vine. During the research the following parameters were tracked such as: yield per vine and hectar, percentage of packed grape and remnants, mechanical analysis of the grape cluster and berry, pressure resistance of the berry, resistance of cluster stem breakage from the berries, chemical analysis etc. The conclusion from the results given from the yield, packed grapes and the quality of the grapes is that best results are shown in the vine with 29 grape clusters.

Key words: grape vine, table grape, Palieri, production and technological characteristics.

Introduction

Republic of Macedonia has good agro ecological conditions for production of table grapes. In near future some activities should be made to increase the areas planted with table grapes known in our country and also introduction of new varieties that will have good perspective in table grape growing. It is also necessary to improve the quality of the table grape with the introduction of suitable agrotechnical measures such as: yield planning with mature pruning, leaving specific numbers of grape clusters per vine on given area and ampelotechnical measures such as: pincage of grape cluster, partial defoliation, use of bioregulators for berry growing and application of modern system of packing.

The subject of this research was the Palieri vine cultivar in Tikvesh wine region. This vine variety originates by crossing the varieties Alphonse Lavallé and Red Malaga. It is on the recommended list of 32 vine varieties for table grape production. Only 30% from the whole area of grape production is with table grapes in Macedonia.

Object and method of work

The research has been conducted on the Palieri vine cultivar in 10 year old vineyard which is in full ripe, on pergola vine training. The distance between rows is 2.4 m and between the vines is 2 m. During the mature pruning, 4 canes is left on each vine with 10 buds per cane and 4 spurs with 2 buds.

This research was conducted in a direction of yield planing on 4 test variants from which one is taken as a main vine or standard.

- Standard (main vine) with leaving of all clusters per grape on each vine,
- Variant 1 with leaving of 19 clusters per grape on each vine,
- Variant 2 with leaving of 24 clusters per grape on each vine,
- Variant 3 with leaving of 29 clusters per grape on each vine,

All Variants and the Standard were equally treated in a matter of irrigation, fertilization, soil cultivation and phyto protection during the period that the research was conducted. In the start of the flowering and pollination phase, 1/3 of the cluster length was removed to all vine variants except for the standard.

During the technological ripeness of the grape: total yield, the percentage of packed grape and remnants are calculated for every variant and standard.

The information about the mechanical contents of the grape cluster and berry, structure of the cluster, mechanical conditions of the grape cluster and berry, sugar content, total acids and pH value of the must are all received in laboratory conditions. The total sugar content in must was measured with Oechsle must meter along with the Dijardin - Salleron table to all variants and standard.

Total acids are given in g/dm^3 calculated with potentiometric titration with the use of bromthymol blue. The neutralization was concluded with 0,1 m NaOH. pH was measured with potentiometric method with pH meter with calomel electrode.

Results and discussion

The yield as a parameter is very strong indicator for the fertility of Palieri variety and it's shown in Table 1.

Table 1. Total yield for Palieri table grape variety

Variants	Packed grape		Remnants		Total
	kg/ha	%	kg/ha	%	kg/ha
1	20.775	95,04	1.084	4,96	21.859
2	23.776	93,88	1.549	6,12	25.325
3	27.163	93,12	2.007	6,88	29.170
Standard	29.188	83,72	5.676	16,28	34.864

The parameters of total yield, percentage of packed grapes and remnant are given for each variant and standard. The highest yield from all test vines has the standard with 34.864 kg/ha, but also with highest percentage of remnants in packed grape with 16,28% or 5.676 kg/ha. Variant 1 has the lowest yield from all, but also with the smallest percentage of remnants in packed grape with 4.96 %. Variant 3 has relatively higher yield with 29.170 kg/ha and also with high percentage of packed grape of 93,12% with just 6,88% of remnants.

Another technological characteristics for table grapes are the dimensions of the cluster and berry shown in table 2.

Table 2. Dimensions of the cluster for Paliery variety

Variant	Length in cm	Width in cm	Size
1	19,9	10,9	-
2	18,9	11,7	-
3	18,2	11,5	-
Standard	25,9	10,1	large

The standard is with the largest dimensions because that the cluster is whole and was not 1/3 removed like the other variants.

The dimensions of the berry for Paliery grape variety for all the researched variants are given in Table 3.

Table 3. Dimensions of the berry for paliery grape variety

Variants	Length in mm	Width in mm	Median value	Length - width in mm
1	27,3	22,8	25,0	1,20
2	26,6	23,1	24,8	1,15
3	26,7	22,6	24,6	1,18
Standard	25,2	22,0	23,6	1,14

The standard has the smallest measured dimensions of the berry of all the researched variants. Variant 1 has the highest values. The results of the mechanical contents are given in table 4.

Table 4. Mechanical content of the cluster for Paliery variety

No:	Elements	V A R I A N T S			
		Standard	1	2	3
1	Cluster weight in g	532,76	612,14	597,62	578,19
2	Cluster remnant in grape bunch g	9,81	11,52	10,41	9,76
3	Berry weight in g	522,95	600,62	587,21	568,43
4	Cluster content indicator	53,31	52,14	56,40	58,24
5	Cluster remnant percentage according to weight	1,84	1,88	1,74	1,69
6	Berry percentage according to weight	98,16	98,12	97,26	98,31
7	Numbers of berries in cluster	70,90	80,28	76,33	78,23
8	Berry indicator	13,30	13,11	12,77	13,53

The highest cluster mass was measured in Variant 1 with 612,14 g and the lowest in standard with 532,76 g. According to the cluster size classification the whole variants including the standard belong to group of very large clusters.

The mechanical content of the berries for the Paliery table grape variety is given in the Table 5.

SECTION 6. VITICULTURE AND WINE PRODUCTION

Table 5. Mechanical content of the berries for the Palieri grape variety

No	Elements	V A R I A N T S			
		Standard	1	2	3
1	Weight of 100 berries in g	902,48	918,65	906,94	910,08
2	Weight of seed in 100 berries in g	9,14	8,76	8,90	8,44
3	Weight of skin in 100 berries in g	32,10	31,08	30,88	31,34
4	Weight of flesh in 100 berries in g	860,94	878,81	867,16	870,30
5	Content indicator in berries	26,82	28,27	28,08	27,77
6	Number of seeds in 100 berries	168	158	164	152
7	Number in seeds in cluster	119,11	126,84	125,18	118,91
8	Weight in 100 seeds in g	5,44	5,54	5,42	5,55
9	Weight in seeds in cluster	6,48	7,03	6,79	6,60
10	Weight in skin in cluster g	22,75	24,95	23,57	24,51
11	Weight in flesh in cluster g	493,72	568,64	556,85	537,32

The variants including the standard have large berries with large values in weight. There are differences between all variants and the standard. The weight of flesh in 100 berries in all variants and standard is higher than 850 grams which gives us conclusion that the berry is large and easily can be noticed which is good indicator for successful sale for this table grape. The structure of the cluster shows the content elements of the cluster shown in %. For Palieri table grape variety is shown in Table 6.

Table 6. Structure of cluster for the Palieri variety

No.	Elements	V A R I A N T S			
		Standard	1	2	3
1	Percentage of cluster remnant	1,84	1,88	1,74	1,69
2	Percentage of skin	4,27	4,07	3,94	4,24
3	Percentage of seeds	1,22	1,15	1,13	1,14
4	Percentage of flesh	92,67	92,90	93,19	92,93
5	Skeleton	6,11	5,95	5,68	5,93
6	Hard remnant	7,33	7,10	6,81	7,07
7	Structural indicator	12,64	13,08	13,68	13,14
8	Theoretical randman	90,96	91,15	91,63	91,35

In the table shown before the whole measured values according to the classification of Prostoserdov are above the median values which gives us to conclude that there is good ratio between the flesh and the other remnant contents in the Palieri table grape variety. In table 7 there are results from the pressure resistance of the berry.

Table 7. Pressure resistance of the berry in g/cm² for the Palieri grape variety

Variant	X - min	X - max	X	CV %
1	1300	3600	2280	26
2	1100	3500	2250	30,3
3	1200	3200	2050	29,4
Standard	1300	2700	1960	23,4

The lowest average value for the pressure resistance is 1960 g/cm², measured in the standard and the highest average value is 2280 g/cm² measured in Variant 1. all of the

variants including the standard have values larger than 1500 g/cm² pressure, which according to the classification belong to very resistant grape varieties that are good for storage and transport. The chemical content of the cluster for the Paliery grape is presented through the sugar content, total acids and pH value of the must shown in Table 8.

Table 8. Chemical content of the cluster for Paliery grape variety

Variants	Sugar g/l	Total acids g/dm ³	pH
1	183	3,30	3,86
2	176	3,45	3,79
3	162	3,67	3,79
Standard	158	3,80	3,69

The lowest sugar content in g/l is measured in the Standard with 158 g/l, but the highest of 183 g/l in Variant 1. The standard has the highest measured values of total acids of 3,8 g/dm³, but the Variant 1 has the lowest of 3,30 g/dm³.

Conclusions

This research for the Paliery grape variety is conducted because there is need for improvement to maintain the quality and maximize the yield for table grape production in our country and wider. Also a multi year strategy is needed for these elements to come true. With a planned number of clusters per vine we could improve the yield with the addition of pincage and partial defoliation and also we will improve quality of the cluster which for a table grape is very important.

The highest yield of 29.170 kg/ha from the conducted research is made in Variant 3. It has very high percentage of packed grape of 93,12% which if we compare with the Standard it has higher yield but the percentage of the packed grape is just 83,72%.

The pressure resistance of the berry for the Paliery grape variety is with high values in all variants. This is a good sign because it also increases the transportability of the grape.

In overall results for total yield, percentage of packed grape, the sugar content, the total acids, the pressure resistance of the berry we can conclude that the best Variant is the Variant 3 with leaving of 29 clusters per grape.

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UDC: 634.862/.863-152.61:631.8(497.715)
Original Scientific Paper**EFFECTS OF FOLIAR FEED ON THE TABLE GRAPES VARIETIES**Prculovski Z.^{1*}, Božinović Z.¹, Boskov K.¹¹Faculty of Agricultural Sciences and Food – Skopje, R. Macedonia

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Abstract

The aim of this research was to establish an influence of foliar fertilizers on yield, weight of bunch, weight of berry, dynamics of aging, transportability and content of sugars and acids at table grape varieties Cardinal, Victoria, Ribier, Afus Ali and M. Italy. We used fertilizers produced by AD Alkaloid – Skopje, according to a program for foliar application in table grape varieties. This research was performed in Gevgelija-Valandovo wine-growing region in commercial plantations of VizbaValandovo winery. The system of growing is pergola. According to the research data, we can conclude that there is a very positive impact of foliar feed up on the yield, weight of the bunch and berry, dynamics of aging, transportability and content of sugar and total acids. The weight of bunch at the treated variant has increased from 9 to 14 %, while the weight of berry at the treated variant has also increased from 8 to 11 %. In the first harvest, the quantity of packaged grapes increased from 6 to 33 %. The total amount of packaged grapes has increased from 7 to 13 %. The sugar's content has increased at the treated variants from 4 to 9 %.

Key words: foliar fertilizers, table grape, yield, weight of the bunch and berry, transportability, chemical composition.

Introduction

Table grape is an important agricultural product which is consumed as a raw product. As of the growing demand and economical worth, its production has been constantly increasing. Table grape production characterizes with specific climate conditions, as well as soil conditions and qualified work force. As of insufficient and expensive work force, activities have been taken for increase of production, quality improvement and work force reduction. To meet the needs of winegrowing science and practice, growth regulators and foliar fertilizers are being increasingly examined and used, because of the positive effects upon the yield and quality of grape. Many authors (M. Colapietra, A. Alexander, G. Ferrara, P. Singram, P.C. Grabu etc.) have examined the effects of using foliar fertilizers for table varieties of vine. In the Republic of Macedonia, the table grape is an important agricultural product. The favorable ecological conditions and human resources have not been used enough in recent years. Great part of the produced table grape has been exported in many countries worldwide. Market competition and costumers' demand have imposed the need of yield and quality increase.

Materials and methods

The examinations have been conducted on the tablegrape varieties Cardinal, Victoria, Ribier, Afus Ali and Muscat Italy, which are cultivated in pergolasystem in Gevgelija – Valandovo wine-growing region. Standard agro-technical and ampelo-technical measures have been used during the production. At each examined variety, a standard has been examined (untreated) and treated grapes with foliar fertilizers according to the program of Alkaloid- Skopje. The following foliar fertilizers have been used:

- Agrosal Bop 8 %
- Agrosal NPK 12-5-7 + ME
- Agrosal NPK 9-0-6 + 10 % CaO + 2 % MgO
- Agrosal NPK 3-20-28 + 3 % EDTA

The influence of the used foliar fertilizers on the weight of bunch, weight of berry, dynamics of aging yield, transportability and content of sugars and acids has been examined.

Examinations of technological characteristics of varieties are conducted according to an elaborated scheme by PHD Zvonimir Bozhinovikj (Ampleography, 2010). The total amounts of sugar in the must (unfermented wine) are determined with Exslov mustmeter. First, the specific weight (thickness) of the must is measured. Then, on basis of the value of the previous measuring, with help of Saleron form, the content of sugar in the must is calculated. The Exslov degrees present the differences between the specific weight of water and the specific weight of the must. The Saleron form gives the volume percent of the sugar i.e. a gram of sugar in 1 liter of must. The total acids (expressed in g/dm³ as the wine acid) are determined with potentiometric titration by using bromothymol blue indicator. The method's principle is based on neutralization of all acids and their salts with diluted 0, 1-M sodium hydroxide, and the quantity of total acids is calculated on basis of the spent solution. Anthocyanins' content is established by using spectrometric methods.

Results and discussion

The gained results for the influence of the used foliar fertilizers upon the weight of bunch are presented in chart 1.

Table 1. Influence of foliar fertilizers upon weight of bunch at table grape varieties

Variety	Standard	Treated	Index
	Weight of bunch	Weight of bunch	Weight of bunch
Cardinal	508	553	109
Victoria	503	573	114
Ribier	385	431	112
Afus Ali	577	635	113
Muscat Italy	622	682	110

The examinations of varieties have shown that the weight of bunch has been increased by 45 g at the variety Cardinal, and by 70 g at the variety Victoria. The average increase of the weight of bunch ranges from 9% at the variety Cardinal, to 14% at the variety Victoria. M. Colapietra (Effect of foliar fertilization on yield and quality of table

grapes, 2006), by using the foliar fertilizers at the variety M. Italia, has gained a bunch weight increase by 77g. The gained results conclude a significant influence of the foliar fertilizers on the weight of bunch. Chart 2 presents the data for the influence of the foliar fertilizers on the weight of bunch at the examined varieties.

Table 2. Influence of foliar fertilizes upon the weight of bunch at the table grape varieties.

Variety	Standard	Treated	Index
	Weight of bunch	Weight of bunch	Weight of bunch
Cardinal	8,0	8,7	108
Victoria	9,4	10,2	109
Ribier	6,3	7,0	111
Afus Ali	6,9	7,6	110
M.Italy	8,0	8,9	111

Having in regard the gained results, an increase of bunch weight at all examined varieties has been concluded. The increase of the weight of bunch ranges from 0, 7 at the variety Cardinal, Ribier and Afus Ali to 0,9 g. at the variety M. Italy. The increase of the bunch's weight ranges from 8 %, at the variety Cardinal, to 11 % at the varieties Ribier and M. Italy. G.Ferrari(Preliminary study on the effects of foliar applications of humic acids on Italia table grape (*Vitisvinifera* L.) cv Italia)by using foliar fertilizers at the variety M. Italy gained an increase of the weight of bunch by 1, 55 g or 14%.

During the production of table grape a very important indicator is the dynamics of grape's aging. It is being insisted more bunches of the vine to mature at the same time. In this way a more efficient harvest can be achieved. Within our examination, we determined the dynamic of aging via the quantity of the packaged grape from the first and second harvest. The gained results for the influence of the used foliar fertilizers are presented in Chart 3.

Table3. Impact of foliar fertilizers upon the dynamic of table grape's aging

Variety	Standard		Treated		Index	
	Iharvest kg/m ²	IIharvest kg/m ²	Iharvest kg/m ²	IIharvest kg/m ²	Iharvest kg/m ²	IIharvest kg/m ²
Cardinal	1,09	0,49	1,15	0,55	106	112
Victoria	1,84	1,2	2,45	0,98	134	82
Ribier	1,63	0,09	1,80	0,06	110	66
Afus Ali	1,60	0,89	1,79	0,95	112	107
M.Italia	1,79	0,82	1,92	1,00	107	122

On basis of the gained results, a positive impact of foliar fertilizers on the dynamics of the grape's aging could be concluded. The quantity of packaged grape in the first harvest has increased from 6% at the variety Cardinal to 34% at the variety Victoria.By using foliar fertilizers at all examined varieties, greater quantity of packaged grape from the untreated variants i.e. the standard has been gained.

The gained results for the influence of the used foliar fertilizes upon the grape yield are presented in Chart 4.

Table 4. The influence of foliar fertilizers on the yield of table grape.

Variety	Standard		Treated		Index	
	Packed kg/m ²	Waste kg/m ²	Packed kg/m ²	Waste kg/m ²	Packed kg/m ²	Waste kg/m ²
Cardinal	1,58	0,37	1,70	0,37	107	100
Victoria	3,04	0,68	3,43	0,55	113	80
Ribier	1,72	0,21	1,86	0,19	108	90
Afus Ali	2,49	0,37	2,74	0,15	110	41
M.Italia	2,61	0,22	2,92	0,13	112	59

The grape yield is divided by the quantity of packaged grape and waste. For the production of table grape the most important thing is the quality of packaged grape. From the gained data and their analyses it could be concluded an increase of the quality of packaged grape from 7% at the variety Cardinal to 13 % at the variety Victoria. The quantity of packaged grape at the treated variants has increased from 0,12 kg/m² at the variety Cardinal to 0,39kg/m² at the variety Victoria. The quantity of grapes in the category waste, by using foliar fertilizers, has been reduced from 20 g/m² at the variety Ribier to 220 g/m² at the variety Afus Ali. At the variety Cardinal, the quantity of grapes from the category waste is identical in both the standard and treated grape. G. Ferrara (Preliminary study on the effects of foliar applications of humic acids on Italia table grape (*Vitis vinifera* L.) cv Italia) by using foliar fertilizers at the variety M. Italy gained an increase of yield by 1, 4 kg per vine.

The transportability of the table grape is a very important technological characteristic. Table grape needs to have better transportability features. The transportability is determined by measuring the reactional strength expressed in g/sm² and the resistance of ripping off, i.e. fixed on the berry at the cap stem expressed in g/berry.

Chart 5 presents the gained results for the impact of the applied foliar fertilizers on the reactional strength and resistance to ripping off of the berry at the examined varieties.

Chart 5. Influence of the foliar fertilizers on transportability at table grape varieties

Variety	Standard		Treated		Index	
	Reactional strengthg/sm ²	Resistance to ripping offg/berry	Reactional strengthg/sm ²	Resistance to ripping offg/ berry	Reactional strengthg/sm ²	Resistance to ripping offg/ berry
Cardinal	2.800	390	3.200	430	114	110
Viktoria	3.160	/	3.800	/	120	
Ribier	2.500	448	2.700	534	108	119
Afus Ali	3.000	460	3.440	470	115	98
M. Italy	2.500	496	3.500	834	140	168

On basis of the gained results and their analyses, a significantly positive influence upon the foliar fertilizers on the strength of the berry and their attachment to the cap stem is concluded. The reactional strength, by using foliar fertilizers has increased from 14% at the variety Cardinal to 40 % at the variety M. Italy. The increase of the reactional strength expressed in g amounts from 200 g/cm² at the variety Ribier to 1.000 g/cm² at the Variety M. Italy. The resistance to ripping off expressed in g/berry has increased from 10 g/berry at the variety Afus Ali to 334 g/berry at the variety M. Italy. The used foliar fertilizers have

the greatest influence in the increase of transportability at the variety M. Italy and the lowest at the variety Afus Ali. Chart 6 presents the data for the content of total sugars in the must at the examined varieties.

Table 6. Influence of the foliar fertilizers on the content of sugars at the table grape varieties

Variety	Standard	Treated	Index
	Sugarg/l	Sugarg/l	Sugarg/l
Cardinal	138	144	1,04
Victoria	/	/	/
Ribier	150	158	1,05
Afus Ali	176	191	1,09
M.Italy	160	167	1,04

On basis of the gained data and their analyses, an increase of the content of total sugar at the variant by using foliar fertilizers, has been concluded. The total amount of sugar has increased from 4% at the varieties Cardinal and M. Italy to 9 % at the variety Afus Ali.

The gained results for the influence of the foliar fertilizers upon the content of total acids are presented in Chart 7.

Table 7. Influence of foliar fertilizers upon the content of total acids at table grape varieties

Variety	Untreated	Treated	Index
	Acids g/l	Acidsg/l	Acids g/l
Cadrinal	6,38	6,68	105
Victoria	4,20	4,30	102
Ribier	4,35	4,43	102
Afus Ali	5,30	5,33	100
M.Italy	4,20	4,35	101

It could be concluded, from the gained results, that the foliar fertilizers do not have a significant influence upon the content of total acids. By using the foliar fertilizers, the content of foliar fertilizers increases from 0, 03 g/l at the variety Afus Ali to 0, 3 g/l at the variety Cardinal.

At the red table wine varieties we have examined the influence of foliar fertilizers on the content of anthocyanin in the skin of the grape. In Chart 8 are presented the gained results for the influence of foliar fertilizers on the content of anthocyanins.

Table 8. Influence of foliar fertilizers on the content of the total anthocyanins (mg/kg grape)

Variety	Standard	Treated	Index
Cardinal	110	126	115
Ribier	191	296	155

The used foliar fertilizers have positive influence on the content of colored material in the skin of berries. The colored material at the variety Cardinal have increased by 16 mg/kg or by 15% , while at the variety Ribier by 105 mg/kg i.e. 55%.

Conclusion

On basis of the gained results for the influence of foliar fertilizers of Alkaloid's program on the production and technological characteristics we adopted the following conclusions:

The applied foliar fertilizers at the examined varieties increase the weight of bunch from 45 to 70 g, and berry's weight from 0,7 to 0,9 g.

The quantity of packaged grapes in the first harvest at the treated variants has increased from 6 to 34 %.

The quantity of the total packaged grape by using foliar fertilizers has increased from 0, 12 kg/m² to 0,39 kg/m². The quantity of grape from the category waste is reduced from 20 to 220 g/m².

Grape transportability of the examined varieties has been improved by application of foliar fertilizers. The reactional strength has increased from 200 to 1000 g/sm², and the attachment of the berry to the cap stem has increased from 10 to 334 g/berry.

The used foliar fertilizers have increased the content of sugar in the must from 4 to 9%, and the one of the total acids to 5 %.

A significant influence the foliar fertilizers have upon the content of anthocyanin in the skin at the varieties Cardinal and Ribier. The treated variants had more intense color, especially the variety Cardinal by 15 %, and the variety Ribier by 55 %.

We encourage the producers of table grape to apply foliar fertilizers according to the program subject of this work, in order to achieve better production results, as well as higher quality of table grape.

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UDC: 634.862/.863-152.61(497.714)
Original Scientific Paper

SOME AMPELOTECHNICAL AND TECHNOLOGICAL CHARACTERISTICS OF THE MOLDAVIA TABLE GRAPE VARIETY IN TIKVES WINE REGION

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Abstract

The purpose of the research was moldavia vine cultivar located in Tikves wine region, purposed for production and consummation of fresh grape in this region. Application of suitable ampelotechnical measures to improve the quality of the table grape is needed such as: yield planning with mature pruning, leaving specific numbers of grape clusters per vine, pincage of grape cluster, partial defoliation, use of bioregulators and application of modern systems of packing. Research was conducted in a direction of yield planning and quality of the grape with leaving 19,24 and 29 clusters per grape on both varieties and leaving of all clusters per grape taken as a standard vine. More parameters were tracked during the research such as: yield per vine and hectare, percentage of packed grape and remnants, mechanical analysis of the grape cluster and berry, pressure resistance of the berry, resistance of cluster stem breakage from the berries, chemical analysis etc. The results and information given from the yield, packed grapes and the quality of the grapes from the different variants that were researched is that best results are found in the Moldavia table grape vine with 29 grape clusters.

Key words: grape vine, table grape, Moldavia, production and technological characteristics.

Introduction

Viticulture is one of the important agricultural activities in Macedonia because there is suitable fertile land for grape growing and other natural conditions for growing of table and wine varieties.

There is long and successful tradition in table grape growing in Macedonian region. Activities are being taken that will result in increasing the area planted with quality varieties of table grape vines that will result in higher yields per hectare.

If we want to improve the quality of the table grape and get higher yields, application of specific ampelotechnical measures such as: pincage of grape cluster, partial defoliation, use of bioregulators for berry growing. Also agrotechnical measures should be used such as: leaving specific number of clusters on the vine per square meter, yield planning with mature pruning as well as the use of modern quality packages for the

packing of the table grapes. The subject of this research was the table grape variety Moldavia in Tikves wine region. This variety originates from Moldova (Kisinev) and it is developed by crossing the SV - 12 - 375 x Guzal Kara by Zuravjev and his associates. It is on the recommended list of 32 vine varieties for table grape production. The area planted with table grape varieties is 30% from the whole grapes production in the country.

Object and method of work

The research has been conducted on the Moldavia vine cultivar in 10 year old vineyard which is in full ripe, on pergola vine training. The distance between rows is 2,4 m and between the vines is 2 m. During the mature pruning, 4 canes is left on each vine with 10 buds per cane and 4 spurs with 2 buds.

This research was conducted in a direction of yield planning on 4 test Variants from which one is taken as a main vine or standard.

- Standard (main vine) with leaving of all clusters per grape on each vine,
- Variant 1 with leaving of 19 clusters per grape on each vine,
- Variant 2 with leaving of 24 clusters per grape on each vine,
- Variant 3 with leaving of 29 clusters per grape on each vine,

All Variants and the Standard were equally treated in a matter of irrigation, fertilization, soil cultivation and phyto protection during the period the research was conducted. In the start of the flowering and pollination phase, 1/3 of the cluster length was removed to all vine variants except for the standard.

During the technological ripeness of the grape: total yield, the percentage of packed grape and remnants are calculated for every variant and standard

The information about the mechanical contents of the grape cluster and berry, structure of the cluster, mechanical conditions of the grape cluster and berry, sugar content, total acids and pH value of the must are all conducted and received in laboratory conditions. Oechsle must meter was used to measure the total sugar content in must along with the Dijardin - Salleron table to all variants and standard.

Total acids are given in g/dm³ calculated with potentiometric titration with the use of bromthymol blue. The neutralization was concluded with 0,1 m NaOH. Potentiometric method was used to measure the pH with the use of pH meter with calomel electrode.

Results and discussion

One of the main indicators for the fertility of the grape is the yield as a parameter and it's shown in Table 1. The yield is an indicator calculated as a total grape mass for a specific grape variety or area. Also another parameters such as: total yield, percentage of packed grapes and remnant are given for each variant and standard in Table 1.

Table 1. Total yield for Moldavia table grape variety

Variants	Packed grape		Remnants		Total
	kg/ha	%	kg/ha	%	kg/ha
1	18.783	94,65	1.062	5,35	19.845
2	22.468	92,72	1.764	7,28	24.232
3	25.854	91,96	2.260	8,04	28.114
Standard	26.499	81,90	5.856	18,10	32.355

From the given results we can conclude that the lowest total yield from all test variants has the Variant 1 with 19.845 kg/ha, and the highest has the Standard with 32.355 kg/ha. Relatively high yield is measured in Variant 3 of 28.114 kg/ha. The percentage of remnants is the lowest in the Variant 1 with 5,35% and the highest in the Standard with 18,10%. In Variant 3 the percentage of remnant is 8,04% and the percentage of packed grape is 91,96%. Another main characteristics of the table grapes are the dimensions of the cluster and berry shown in table 2.

Table 2. Dimensions of the cluster for Moldavia variety

Variant	Length in cm	Width in cm	Size
1	13,8	12,6	-
2	14,2	11,9	-
3	14,3	11,4	-
Standard	23,8	10,5	large

The largest dimensions of the cluster is measured in the Standard because that the cluster is whole and was not 1/3 removed like the other variants. The dimensions of the berry for Moldavia grape variety for all the researched variants are given in Table 3.

Table 3. Dimensions of the berry for Moldavia grape variety

Variants	Length in mm	Width in mm	Median value	Length - width ratio in mm
1	24,3	19,1	21,7	1,27
2	23,5	18,7	21,1	1,26
3	23,6	18,4	21,0	1,28
Standard	22,5	17,8	20,1	1,26

There are specific differences in length and width of the berries between the variants and the standard. The standard has the smallest dimensions of the berry in comparison with the other researched variants. The results of the mechanical contents are given in Table 4.

Table 4. Mechanical content of the cluster for Moldavia variety

No:	Elements	V A R I A N T S			
		Standard	1	2	3
1	Cluster weight in g	422,05	466,71	449,71	428,95
2	Cluster remnant in grape bunch g	8,23	10,38	9,13	9,71
3	Berry weight in g	413,82	456,33	440,58	417,24
4	Cluster content indicator	50,28	43,96	48,25	42,97
5	Cluster remnant percentage according to weight	1,95	2,22	2,03	2,26
6	Berry percentage according to weight	98,05	97,78	97,97	97,74
7	Numbers of berries in cluster	82,10	90,57	87,14	80,86
8	Berry indicator	19,45	19,40	19,37	18,85

From the given results we can conclude that highest cluster mass was measured in Variant 1 with 466,71 g and the lowest cluster mass in the standard. According to the cluster size classification the whole variants including the standard have weight more than 400 grams

and belong to a group of very large clusters. The mechanical content of the berries for the Moldavia table grape variety is given in the Table 5.

Table 5. Mechanical content of the berries for the Moldavia grape variety

No	Elements	V A R I A N T S			
		Standard	1	2	3
1	Weight of 100 berries in g	602,94	672,12	618,38	610,74
2	Weight of seed in 100 berries in g	11,10	10,44	10,06	11,62
3	Weight of skin in 100 berries in g	28,82	29,76	35,10	35,98
4	Weight of flesh in 100 berries in g	563,02	631,92	573,22	563,14
5	Content indicator in berries	19,54	21,23	16,33	15,65
6	Number of seeds in 100 berries	188	192	176	198
7	Number in seeds in cluster	154,34	173,89	172,42	160,10
8	Weight in 100 seeds in g	5,90	5,44	5,71	5,86
9	Weight in seeds in cluster	9,11	9,45	9,72	9,39
10	Weight in skin in cluster g	23,66	26,95	30,58	29,09
11	Weight in flesh in cluster g	381,05	419,93	400,28	378,76

There are differences in values between the variants and the standard . Values measured above 600 grams are found in all the variants and the standard in the elements of weight of 100 berries in g. The structure of the cluster that shows the contents of the cluster is measured in %. For the Moldavia table grape variety it is shown in Table 6.

Table 6. Structure of cluster for the Moldavia variety

No	Elements	V A R I A N T S			
		Standard	1	2	3
1	Percentage of cluster remnant	1,95	2,22	2,03	2,26
2	Percentage od skin	5,60	5,77	6,80	6,78
3	Percentage od seeds	2,15	2,02	2,16	2,19
4	Percentage of flesh	90,30	89,99	89,01	88,73
5	Skeleton	7,55	7,95	8,83	9,04
6	Hard remnant	9,70	10,01	10,99	11,23
7	Structural indicator	9,31	8,99	8,10	7,90
8	Theoretical randman	88,54	87,99	87,20	86,72

From the results given we can conclude that all of the variants and the standard have high percentage of flesh. The mechanical characteristics of the berries are presented through their pressure resistance in Table 7.

Table 7. Pressure resistance of the berry in g/cm² for the Moldavia grape variety

Variant	X - min	X - max	X	CV %
1	1000	2200	1490	19,3
2	1100	2500	1710	23,4
3	1000	1900	1390	14,8
Standard	600	1900	1160	25,8

The lowest average value for the pressure resistance of the berry is measured in the standard. It can be concluded that all variants have relatively high values for pressure

resistance. The chemical content of the cluster for the Moldavia grape is presented through the sugar content, total acids and pH value of the must shown in Table 8.

Table 8. Chemical content of the cluster for Moldavia grape variety

Variants	Sugar g/l	Total acids g/dm ³	pH
1	221	4,92	3,43
2	214	5,10	3,51
3	200	5,35	3,30
Standard	187	5,52	3,36

The lowest sugar content in g/l is measured in the Standard with 187 g/l, but Variant 1 has the highest sugar content of all with 221 g/l. The contents of total acids are highest in the Standard with 5,52 g/dm³ and lowest in Variant 1 with 4,92 g/dm³.

Conclusions

The subject of our research was the Moldavia grape variety where with leaving of specific numbers of cluster and further treatment with pincage we can improve the quality of the grape and increase the total yield. These improvements will give further advantage and competitive sales in the table grape market.

Variant 3 has the highest measured yield of 28.114 kg/ha with 91,96 % of packed grape compared with the Standard which has 34.864 kg/ha but with much lower percentage of packed grape of 83,72%. The pressure resistance of the berry for the Moldavia grape variety is with high values in all variants and the standard. This is a good sign because it also increases the transportability of the grape. While reducing clusters, the transportability increases. The sugar content measured in all Variants is higher compared with the Standard. The cluster reduction increases the sugar concentration in the grape.

Summarizing the overall results for total yield, percentage of packed grape, the sugar content, the total acids, the pressure resistance of the berry we can conclude that the best variant is the Variant 3 with leaving of 29 clusters per grape.

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UDC: 634.8.03 (497.7)
Original Scientific Paper

**AMPELOGRAPHIC DESCRIPTION OF THE GROUP PHENOTYPIC
PROPERTIES IN SOME CULTIVARS OF GRAPEVINE, GROWN IN R.
MACEDONIA, IN ADDITION TO METHODS FOR DETERMINING OF
THEIR ORIGIN**

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Abstract

In every country in the viticultural production, are grown different cultivars grapevines. They may be autochthonous (domestic and domesticated), introduced from different near and distant countries, selected cultivars, different species of the genus *Vitis* etc. Identification of different varieties consists of several related methods which are complementary each other (DNA identification ampelografija, ampelometrija, centers of origin and so on.). In assortment of R. Macedonia are represented different varieties from different geographical climes and with different backgrounds. Therefore, the selective viewpoint in the this examination, four different cultivars (agadaj, sangiovese, chaush and smederevka) are analyzed, as typical representatives from different regions and they are grown in vineyards of our country. In these cultivars are described group of several primary and secondary characteristics associated with fertilization, productivity and quality. The description is made according to OIV Code system of descriptors. The cultivars are not obtained by crossing, but originated and taxonomic belong to different ecological - geographic groups and therefore they are suitable for this type at a comparable examination. Some of the cultivars are grown in large percentage, and some are locally with a small percentage. The aim of this examination is to make a comparison between the cultivars with the method of identification (ampelographic description) and display their characteristic differences in the anatomical structure. Based on the observed differences in the description of a particular group of properties and according to their centers of origin, these cultivars are classified as typical representatives of their ecological - geographical group. Mostly, phenotypic characteristics differ from the first (primary) list of descriptors.

Key words: identification, ampelographic description, OIV code, descriptors, centers of origin, primary and secondary characteristics, autochthonous cultivars

Introduction

There are more options for proving the origin of grapevine cultivars. One reliable method is ampelographic description, according to which based on certain selected characteristics, can be identified one variety and determine its affiliation to a particular ecological-

geographic group. The reliability of determining the origin and belonging to grapevine cultivars according to ampelographic description to a group of properties is high. Phenotypic description of certain specific anatomical features, in specific segments of the plant, in a certain phenological development, that represents correct data on the grouping of grapevine cultivars in certain groups, according to the similarity of those properties. The accuracy of ampelographic description depends on several factors (age of the observed organs stage of the plant, environmental conditions, etc).

But, for greater precision in determining the origin, ampelographic description is necessary to be applied in combination with other identification methods, such as DNA - analysis, history, centers of origin and so on. DNA - identification is characterized by high accuracy and provides reliable data on genetic predisposition and genetic capacity of varieties.

In this paper we decided to use ampelographic description of the examined varieties. It is aimed to indicate in detail the differences between the cultivar and expressed precision be confirmed their origin and their belonging in a certain ecological and geographical group.

Material and methods

Four varieties of grapevine - smeredevka, chaush, agadaj and sangiovese were examined; they are randomly selected and originate from different ecological and geographical areas. They are represented in more vineyards in R. Macedonia. Examined cultivars are grown in crop collections of the Institute of Agriculture in Skopje and some limited vineyards – Tikves, Gevgelija-Valandovo wine region. The examinations were performed in the period from 2005 to 2014.

The cultivar agadaj is white table cultivar that belongs to the group (convarietas) *orientalis*, subgroup (subconvarietas) *antasiatica*. In our country is represented on limited areas in Gevgelija-Valandovo, Veles and Tikves wine region. The cultivar sangiovese is a cultivar for production of red wines, that belongs to convarietas *occidentalis*, subconvarietas *gallica*, here had been introduced in 2000. The cultivar chaush is white table cultivar that belongs to convarietas *pontica*, subconvarietas *balcanica*, domesticated (local) cultivar, is represented on limited areas and a small percentage in our vineyards. The cultivar smeredevka is white wine cultivar, belongs to convarietas *pontica*, subconvarietas *balcanica*, is grown a long period and is represented in a large percentage of our vineyards.

The morphological characteristics of the varieties are examined according with ampelographic description lists of primary and secondary OIV Code descriptors. The list was proposed by the EU-PROJECT GENRES 081 - 09/2001. Additionally recent edition of 2009 was used. The results of the ampelographic description are obtained by comparing the average received from 10 representative samples of an anatomical part of the plant with standard cultivars or species. So is formed a score of 1 to 9, depending on which evaluations are based OIV Code descriptors. Every characteristic is marked with a code, the category is marked with words, and the assessment is marked with numbers. For proving of belonging and origin was elaborated ecological and geographical classification of cultivars according to A. M. Negrul. The germination of the pollen was examined *in vitro*, with planting pollen grains in fertile base of 15% saccharose solution, in a preparation hanging drop and with keeping it in a thermostat on a temperature of 21°C. After that the germinated pollen grains were counted and photographed under a

microscope. Fresh pollen treatment with 1% orcein solution was made for determining of alive pollen.

Results and discussion

In this paper, the processed cultivars were examined group of anatomical and morphological characteristics that are significant from genetic and breeding aspect. Also, these properties are important to confirm the origin of cultivars. For proving of belonging and origin was elaborated ecological and geographical classification of cultivars according to A. M. Negrul. This classification is based on the close genetic relationship between the cultivars from the same geographic area.

The type of the flower and the characteristics of bunch, berry, seeds and length of peduncula, they are characteristic that indicate how their fertilization, the quality of the grapes and their origin.

From the discussion of the results obtained separately by varieties, can be seen that the cultivars – agadaj, sangiovese and smeredevka have morphologically and functionally hermaphroditic flower and the cultivar chaush has morphologically hermaphrodite and functionally female flower with stamens bent down.

According to the size of bunch and berries and their shape has no significant deviations from their classification. The cultivars – agadaj and chaush as table grape cultivars have larger berries and the cultivars – smeredevka and sangiovese as wine grape cultivars have smaller berries. The cultivars - agadaj, chaush and smeredevka have yellowish to green color of skin and the cultivar sangiovese has dark blue color of skin. All varieties are characterized by medium density to loose density of the bunch which means that they are well fertilized. Also the cultivar chaush indicates good fertilization although she is functionally female cultivar and she used other pollinators. The length of the peduncle of the bunch the cultivar chaush is evaluated as a long peduncle, and other cultivars have medium length. Also, over the maturation, the cultivar chaush indicates specific, poorly expressed vanilla flavor. In all cultivars have normally developed seeds. The cultivar agadaj has large seeds with a long beak. The cultivar sangiovese has small seeds with a small beak. The cultivars smeredevka and chaush have medium sized seeds.

Table 1. Primary ampelographic descriptors with assessing

OIV Code N°	Descriptor	Characteristics	Cultivar			
			Agadaj	Sangiovese	Caus	Smederevka
OIV 151	Inflorescence:	sex of flower	3	6	4	3
OIV 202	Bunch:	length	7	5	5	5
OIV 208	Bunch:	shape	1-3	2	2	2
OIV 209	Bunch:	number of wings	2	2	2	2
OIV 220	Berry:	length	7	5	5-7	5-7
OIV 221	Berry:	width	7	3-5	5-7	5
OIV 223	Berry:	shape	2	2-3	6-2	3
OIV 225	Berry:	color of skin	1	6	1	1
OIV 236	Berry:	particular flavor	1	1	5	1
OIV 241	Berry	presence of seeds	3	3	3	3

SECTION 6. VITICULTURE AND WINE PRODUCTION

In terms of percentage of alive pollen the cultivar chaush has the lowest percentage - 20.53 and the cultivar agadaj has the highest percentage - 90.16. The percentage of germination of pollen is in cultivar chaush - 0.00 (sterile pollen) and highest percentage has the cultivar agadaj - 80.30 (agadaj has good self-pollination). In terms of these two parameters, there are major differences among the examined cultivars, which shows the high coefficient of variation (CV). Percentage of alive pollen and percentage of pollen germination are shown in Chart. 1

Table 2. Secondary ampelographic descriptors with assessing

OIV Code N°	Descriptor	Characteristics	Cultivar			
			Agadaj	Sangiovese	Caus	Smederevka
OIV 204	Bunch:	density	5-3	5	5-3	5-3
OIV 206	Bunch:	length of peduncle	5	5	7	5

Table 3. Percentage of alive pollen and percentage of pollen germination

Cultivar	Year	Alive pollen %	CV %	Pollen germination %	CV %
Agadaj	2005/2014	90,16	47,27	80,30	66,71
Sangioveze		83,54		76,95	
Caus		20,53		0,00	
Smederevka		85,72		78,48	

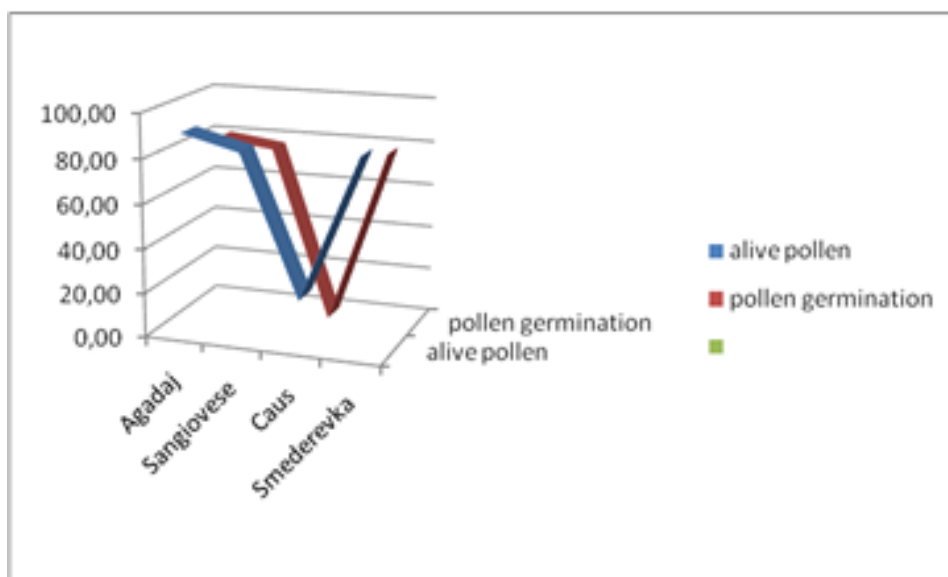


Chart. 1

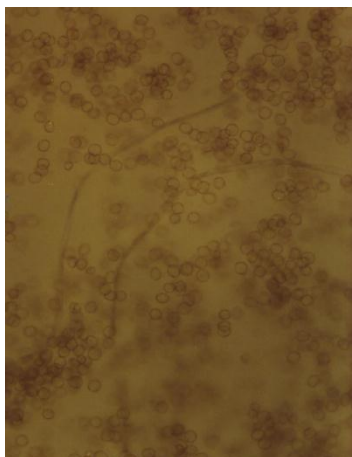


Figure 1. Fertile pollen in the cultivar smeredevka



Figure 2. Sterile pollen in cultivar chaush



Figure 3. Chaush



Figure 4. Smederevka



Figure 5. Sangiovese

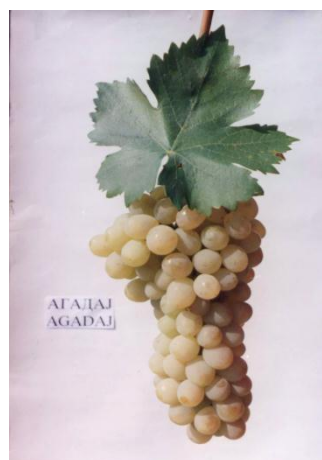


Figure 6. Agadaj

Conclusions

From the obtained results can be concluded:

- According to the description of the size and shape of bunch and berries, examined cultivars are typical of their ecological-geographical groups (NEGR.).
- The cultivar agadaj is characterized by a large cylindrical bunch with large berry, berry often has a round shape. The characteristics are typical of the Eastern group of cultivars.
- The cultivar sangiovese has medium to small conical bunch, medium to small spherical berries, they are typical of the Western group of cultivars.
- The cultivars chaush and smeredevka have medium conical bunches and medium berries with a round shaped which is typical for the Balkan group of cultivars.
- By comparing the type of flower and pollen fertility can say that in Eastern and Balkan group of cultivars frequently found functionally female flowers and sterile pollen, unlike Western group of cultivars.
- The cultivar agadaj has the largest seeds with a long beak (typical of the Eastern varieties). The cultivar sangiovese has small seeds with a small beak (typical of Western varieties). The cultivars smeredevka and chaush have medium seeds (Balkan varieties).
- Examined cultivars are typical representatives of their centers of origin or their ecological and geographic groups (according Negrul) and they can be used as a benchmark in the comparative tests.

As a recommendation, though in some trials has already made molecular analysis of the origin of these cultivars again is necessary to make DNA - identification to complement ampelographic description.

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ECOLOGICAL CONDITIONS USED TO PREDICT THE BEGINNING OF FLOWERING OF 'ŽILAVKA' VARIETY IN THE MOSTAR WINEGROWING REGION

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Abstract

Flowering is one of the most sensitive phenophases of grapevine, and it can be largely affected by climatic conditions prevailing during the period. Temperature conditions that precede the beginning of flowering are of great, if not critical, importance to the onset of this phenophase. Temperatures above 15°C favor pollination and fertilization. The beginning of grapevine flowering is of utmost importance to defining the dynamics of major cultural and canopy managements practices in the vineyard. This paper presents a model for predicting the beginning of flowering in the variety 'Žilavka' based on the analysis of temperature conditions in the Mostar winegrowing region. To construct the model to be used for predicting the onset date of flowering in 'Žilavka' depending on the temperature sum for the month preceding flowering, the Mostar Weather Station data for the period 2002- 2010 were used. Based on the number of days between first budbreak and onset of flowering (Y) and the value of the F coefficient (x) obtained from the ratio of the temperature sum for the month preceding flowering to the number of days between first budbreak and onset of flowering, the best-fit regression equation was calculated. This quadratic-type equation is given as: $Y = 143.01 + (-16.55x) + 0.70x^2$; $S_y = 2.69$; $r = -0.96^{**}$. Testing the significance of differences between the actual observed number of days from first budbreak to onset of flowering and the predicted number for the same period calculated using the above equation shows no significant differences, thus suggesting that this function can be successfully applied as a model to predict the beginning of flowering for 'Žilavka' in the Mostar winegrowing region.

Key words: grapevine, variety, temperature, flowering

Introduction

During the growing season, the grapevine goes through six phenological phases viz. bleeding, budbreak, flowering and fertilization, berry growth, berry ripening, and shoot ripening and leaf fall. The beginning of flowering is marked by the shedding of the cap which is preceded by abscission zone formation at the petal base. In 'Žilavka', when released from the anthers, the pollen is intensely dispersed under the cap and abundantly found on the anthers themselves, on the stigma and other parts of the flower (Jovanović-

Cvetković, 2013). Grapevine flowering phenology is very sensitive to climatic conditions prevailing during the period. Under particularly favorable environmental conditions, grapevine flowering is typically completed in 5 to 7 days, whereas its average length is 7 to 14 days. In contrast, under less favorable climatic conditions, flowering can extend for some 20 or more days. The beginning of flowering is primarily dependent on temperature during the preceding period (Jovanović-Cvetković, 2013). Therefore, the length of the flowering phenophase in grapevines is apparently directly dependent on the mean daily air temperature. Apart from temperature, flowering is governed by other factors such as light, soil fertility and vine vigor (Haba, 1989). The determination of the onset of flowering is mostly based on ecophysiological investigations and the fact that the flowering period is mostly preceded by or that the flowering phenophase coincides with the beginning of inflorescence differentiation in the winter bud. The period between first budbreak and the onset of flowering shows an average of 30 to 60 days, depending on variety and, naturally, temperature (Mijatović et al., 2012). The length of the period generally depends upon the amount of heat. The average heat sum required for the grapevine to start flowering after the onset of budbreak is about 380°C (Burić, cit. Mijatović, 1990; Mijatović et al., 2012). Predicting the onset date of grapevine flowering is important in terms of the planning and use of appropriate cultural and canopy management practices in the vineyard.

Material and methods

Monitoring of temperature conditions between the beginning of the growing season and the onset of flowering, and phenological observations for 'Žilavka' were carried out during the period 2002-2010 in the Mostar region. The construction of the model used for predicting the onset date of flowering in 'Žilavka' depending on the temperature sum for the month preceding flowering was based on the data provided by the Mostar Weather Station. The following data were used for the research: a) mean daily air temperatures and b) onset dates of budbreak and flowering phenophases for 'Žilavka'.

For convenience of calculation, the onset dates of budbreak and flowering were converted to days using the conversion table that converts the number of days to dates and vice versa (Mijatović and Jovanović-Cvetković, 2007). To construct the model for predicting the beginning of flowering in 'Žilavka', the following analyses were conducted across years:

1. Determination of the number of days between the onset dates of budbreak and flowering for each year separately (Table 1).
2. Determination of the temperature sum based on mean daily temperatures for the month preceding the onset of the flowering phenophase (Table 2).
3. Calculation of the F coefficient (flowering coefficient) as the ratio of the temperature sum to the number of days between the onset dates of budbreak and flowering (Table 2).
4. Determination of dependence between the number of days from first budbreak to onset of flowering and the F coefficient, as expressed by a regression equation.

The regression equation was used to calculate the predicted number of days between first budbreak and flowering and compare it with the actual observed number of days between the two dates, with significance assessed by a paired difference test (Table 3).

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Table 1. Onset dates of budbreak and flowering, and number of days between the two dates for 'Žilavka'.

<i>Year</i>	<i>Onset of budbreak</i>	<i>Onset of flowering</i>	<i>No. of days between the onset dates of budbreak and flowering</i>
2002	93	158	65
2003	91	156	65
2004	98	162	64
2006	90	145	55
2007	84	130	46
2008	85	132	47
2009	86	142	56
2010	90	145	55
Average	90	146	57

Table 2. Temperature sum for April and F coefficient values

<i>Year</i>	<i>Temperature sum for April</i>	<i>„F” coefficient value</i>
2002	429	6.60
2003	405	6.23
2004	438	6.84
2006	450	8.18
2007	513	11.15
2008	420	8.94
2009	468	8.36
2010	444	8.07
Average	445.9	8.05

Table 3. Observed and predicted numbers of days between first budbreak and onset of flowering

<i>Year</i>	<i>Observed number of days between first budbreak and onset of flowering</i>	<i>Predicted number of days between first budbreak and onset of flowering</i>
2002	65	64.21
2003	65	67.02
2004	64	62.49
2006	55	53.38
2007	46	45.35
2008	47	50.90
2009	56	53.49
2010	55	54.96
Average	56.62	56.47
Sx	2.52	2.45
Vk	14.36	12.25

$t_{\text{calc}}=0.043$; $t_{\text{tab}0.05}=1.76$; $0.01=2.62$

Results and discussion

Analysis of the onset dates of budbreak and flowering

The stage of flowering under the climatic conditions of Mostar begins towards the end of May or early in June. The analysis of the onset of flowering in 'Žilavka' under the climatic conditions of the Mostar winegrowing region during the period 2002-2010 at the

Aluminium Smelter location shows that flowering onset ranged from 10 May (2007) to 11 June (2004), with the mean onset date of 26 May. The number of days between first budbreak and onset of flowering was in the range of 46 (2007) to 65 (2003), giving an average of 57, which is in agreement with the reports of Burić (1981).

Analysis of temperature sums

The analysis of temperature sums for April (Table 2) shows the range between 405.0 °C in 2003 and 513.0 °C in 2007. The data reveal that the average temperature sum for April under the conditions of the Mostar winegrowing region was rather high i.e. 446.0 °C compared to the corresponding data for the 1972-1986 period, when it was as low as 380 °C (Mijatović, 1990).

Analysis of F coefficient values

The F coefficient values given in Table 2 range from 6.23 (2003) to 11.15 (2007) for 'Žilavka' under the conditions of the Mostar winegrowing region. The average value during the observation period was 8.05. In 2003, the number of days between first budbreak and onset of flowering was the highest (65), whereas the temperature sum for April was rather low 405.0 °C, and the F coefficient value 6.23. However, in 2007, the number of days between the onset dates of budbreak and flowering was 46, the temperature sum as high as 513.0 °C, and the F coefficient value 11.15. The data obtained suggest the following pattern: the higher the F coefficient value, the smaller the number of days between the onset dates of budbreak and flowering, and the higher the F coefficient value if the temperature sum of the month preceding flowering is higher. The F coefficient values as the independent variable (x) and the number of days between first budbreak and onset of flowering as the dependent variable (Y) were used for calculating the regression equation i.e. as parameters of the flowering prediction model. The regression equation is quadratic-type in the following form:

$$Y = 143.01 + (-16.55x) + 0.70x^2 \quad S_y = 2.69 \quad r = 0.96^{**}$$

Testing of the significance of the correlation coefficient shows a highly significant relationship between the number of days from first budbreak to the onset of flowering and the F coefficient. Noticeably, this equation can serve as a model for estimating the number of days required for flowering to begin after the observed onset of budbreak.

Comparative analysis between the observed and predicted numbers of days between the onset dates of budbreak and flowering

Table 3 presents data used for the comparative analysis on the applicability of the model proposed i.e. data on the observed number of days and the predicted number of days obtained from the regression equation. The difference test shows no significant difference at $p = 0.01$ between the observed number of days from first budbreak to onset of flowering and the predicted number of days obtained by the regression equation - model for the same period i.e. the test suggests that the regression equation completely exhibits the dependence of the onset date of flowering in 'Žilavka' on the preceding month's temperature conditions, thus showing that this function can serve as a model for predicting the onset of flowering. This model can be used to estimate the onset of flowering in other

varieties or in the same variety at other locations by simply calculating regression equation values based on appropriate data.

Conclusion

The model used for predicting the onset date of flowering in grapevines depending on the temperature sum of the month preceding flowering was constructed based on the Mostar Weather Station data for the period 2002-2010 and the phenological observations for the variety 'Žilavka' under the same environmental conditions. Based on the data on the number of days between first budbreak and onset of flowering (Y), and the value of the F coefficient (x) obtained from the ratio of the temperature sum of the month preceding the beginning of flowering to the number of days between first budbreak and onset of flowering, the best-fit regression equation was calculated. Testing the significance of differences between the observed number of days from first budbreak to onset of flowering and the predicted number of days for the same period obtained by the regression equation shows no significant difference between them, suggesting that this function can be used as a model for predicting the onset of flowering in 'Žilavka' at the Mostar location. Furthermore, the same principle may also be used for predicting flowering date of other varieties and in other climates.

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SECTION 7. FRUIT GROWING

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RESULTS OF HYBRIDIZATION BETWEEN SOUR AND SWEET CHERRY (*P. cerasus* L. × *P. avium* (L.) L.)

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Abstract

Interspecific hybridization in fruit-growing has been successfully applied in breeding new rootstocks and cultivars. Although the first breeding results in this direction have had transient success, as a result of the recent breeding programmes, a number of different rootstocks, mainly for stone fruit species, have already been implemented and approved in practice. The cherry breeding programme of the Fruit-Growing Institute – Plovdiv was initiated in 1987. Rendering an account the vigorous growth of cherry trees, one of the programme aims was the establishment of rootstocks inducing moderate and poor growth of the grafted cultivars, displaying a resistance to abiotic and biotic stress factors. The sour cherry cultivars ‘Schattenmorelle’, ‘Polevka’, the cultivar of an interspecific origin ‘Érdinagygyümölcsű’ (M-15) and the clone of ‘Zhukovskaya’ cultivar, named ‘Ranna Zhukovskaya’, were included in the interspecific hybridization programme as mother parents. The sweet cherry cultivars ‘Stella’, ‘Compact Stella’ and ‘Compact Van’ were used in the programme only as pollen parents. 124 hybrids from five parent combinations had been grown until entering the fruiting stage. 31 hybrids were grown from the second largest population of the parent combination (‘Polevka’ × ‘Compact Van’), from which, after many-year observations, the hybrids No. 20-181 and No. 20-192 were selected. They are characterized by poor growth, distinct drought resistance and resistance to diseases and pests.

Key words: sweet cherry (*Prunus avium*), sour cherry (*Prunus cerasus*), interspecific hybridization, rootstock breeding.

Introduction

Interspecific hybridization occupies a prominent place among breeding and genetic studies. It has been attracting the attention of a large group of researchers from different parts of the world for many years. The vast interest in this area of science is determined by the great opportunities for "construction" of new genetic types and for synthesis of new interspecific hybrids with wide potential for variability and selection of plant forms that combine characteristics of different taxa, desired by the researcher.

Interspecific hybridization in fruit-growing has been applied with increasing success in breeding rootstocks and cultivars. In the recent years, the number of rootstocks obtained in result of interspecific hybridization with the participation of different *Prunus* species, including ornamental ones, used for stone fruit species, has been increasingly growing. Although the first breeding results in this direction have had transient success, a number of

different rootstocks mainly for stone fruit species, have already been implemented and approved in practice in result of the recent breeding programs.

A huge work in that aspect has been carried out at the Grand Ferrad Research Centre of Fruit-Growing in France. The rich hybrid fund enabled the researchers to establish a diverse group of interspecific hybrids, most of them being infertile. However, it was not an obstacle to use some of them as rootstocks, as they were easily propagated by vegetative methods (Enikeev, 1969).

In the 50ies of 20 c., at the East Malling Research Station in England, the Hybrid No. 21 of a weak growth rate was established by crossing the species *P. avium* and *P. pseudocerasus*, which was later spread as a vegetative cherry rootstock named 'Colt' (Webster, 1989).

Interspecific hybridization for establishing rootstocks for cherries of a weak growth rate was conducted in Germany, using the species *P. avium*, *P. cerasus*, *P. canescens* and *P. fruticosa*, by carrying out reciprocal crossings between each species with the other three. In Oregon, USA, the semi-dwarfing cherry rootstocks 'Brookforest' and 'Brookgrove' were bred by crossing the species *P. avium* and *P. mahaleb*, introduced later in France under the names 'Maxma 14' and 'Maxma 97', respectively. In 1962 the Experimental Station in Gembloux, Belgium started a breeding programme for developing dwarfing vegetative rootstocks for cherry. Out of the three established rootstocks, 'Inmil' (GM9) proved to be the weakest in growth. It was a result of hybridization between the species *P. incisa* and *P. serrula* (Trefois, 1989; Druart et Trefois, 1991).

The cherry rootstock of Gisela series established in Germany, are also of an interspecific origin. 'Gisela 1' and 'Gisela 10' were obtained by crossing the species *P. fruticosa* × *P. avium*. 'Gisela 4' was developed by reciprocal crossing of *P. avium* × *P. fruticosa*. The rootstocks 'Gisela 5', 'Gisela 6' and 'Gisela 7' were the result of hybridization between the species *P. cerasus* and *P. canescens* (Edin et al., 1997; Lugli et al., 2005). The first information about interspecific hybridization in fruit tree species in Bulgaria was provided by Georgieva (1964). According to her, the activities were carried out in several research areas, the hybridization among the species of *Prunus* genus being one of them. According to Panov et al. (1965), breeding activities on interspecific hybridization, including with representatives of *Prunus* genus, started at the Experimental Station in Kostinbrod in the period 1953-1956.

Only 3 hybrid plants were obtained from the parental combinations *P. avium* × *P. cerasus*. The trees had the normal habit of sweet-and-sour-cherry hybrids. The reciprocal combination *P. cerasus* × *P. avium* proved to be considerably more prolific concerning the number of the obtained hybrid plants. 151 plants were obtained from that combination, two of which having large fruits of very good taste and technological qualities.

Breeding activities for establishing interspecific and intergeneric hybrids was initiated at the Fruit-Growing Institute – Plovdiv in 1964 by Baev (1971; 1973; 1973a; 1974; 1978). Cultivars and wild species of *Malus*, *Pyrus*, *Cydonia* and *Prunus* genera were included in the hybridization process. Over 1300 hybrid plants of F1 and F2 generations were established.

Almost in the same period Dabov (1972, 1974, 1975, 1983, 1983a) conducted detailed genetic and breeding studies on the mechanism of inheritance of the resistance to the agent causing the powdery mildew disease in peach (*Sphaerotheca pannosa* (Wallr.) Lev.). In his breeding activities, totally devoted to the development of new peach and nectarine cultivars resistant to powdery mildew and leaf curl disease *Thaphrinadeformans*

(Fusk.)Tul.), the author included representatives of the species *Prunus persica*, *P. ferganensis* and *P. davidiana*. Five peach cultivars for fresh consumption and two cultivars for processing were established.

The cherry breeding programme of the Fruit-Growing Institute in Plovdiv started in 1987. Rendering an account of the vigorous growth rate of the cherry trees, one important aim of the programme was the establishment of rootstocks inducing moderate to weak growth of the trees of the grafted cultivars, resistant to abiotic and biotic stress. The available diverse cherry genetic fund, the rich hybrid fund of F1 and F2 generations and the established breeding values of a number of sweet and sour cherry cultivars and combinations as donors of concrete characteristics, were a sound basis for further success in breeding activities on cherry at the Fruit-Growing Institute – Plovdiv (Zhivondov, 1994; Zhivondov et al., 2000; Zhivondov, 2012).

The present experiment had the aim of making a brief analysis of the results of hybridization between sour and sweet cherry, as well as of presenting the major pomological characteristics of the obtained rootstocks No. 20-181 and No. 20-192 for sweet and sour cherry cultivars, characterized by a weak growth rate.

Materials and Methods

In 1987 a new stage of the breeding activities on interspecific hybridization started at the Fruit-Growing Institute – Plovdiv. The hybridization plan of the programme was developed on the basis of many-year pomological studies on the major characteristics of large groups of stone fruit cultivars and wild forms grown in collection plantations. Taking into account that the possibilities of intraspecific hybridization have been exhausted to a great extent, cultivars and forms of the species *Prunus avium* (L.) L. and *Prunus cerasus* L. of a weak growth rate and compact habit were selected. The seedling rootstocks of the species *P. mahaleb* and *P. avium*, still widely used in a number of countries, induce vigorous growth and the formation of large tree crowns of the grafted cultivars that makes it difficult to establish modern plantations of an intensive type, in which the growing practices are much easier. On the other hand, a large number of the developed and introduced into practice dwarf rootstocks for sweet and sour cherry have many disadvantages, making them quite more pretentious to modern technologies for establishing and growing new plantations. Due to those complex reasons, one of the major aims in the breeding programme on remote hybridization of the Fruit-Growing Institute in Plovdiv was the development of rootstocks for sweet and sour cherry cultivars, characterized by a weak growth rate, resistant to biotic and abiotic stress factors, with a good plasticity and compatibility with the grafted cultivars, suitable for the establishment of dense modern plantations in different soil types.

The sour cherry cultivars ‘Schattenmorelle’, ‘Polevka’, the hybrid cultivar ‘Erdinagygyümölcsü’ (M-15) and the selected clone ‘RannaZhukovskaya’ were included as mother parents in the interspecific hybridization programme between sour and sweet cherry. The sweet cherry cultivars ‘Stella’, ‘Compact Stella’ and ‘Compact Van’ were used in the programme only as pollen parents. The classical method of sexual hybridization was applied. Hybrid evaluation and selection were conducted by visual observations at two levels: the first one – selection in the breeding nursery and the second one – selection in the breeding plantation. All the hybrids were grown under non-irrigation conditions against

a natural infectious background, without any plant protection activities applied, with the aim of evaluating their drought resistance and susceptibility to diseases and pests.

Results and discussion

Hybridization was performed in 1991 and in the next two years the hybrids were grown and evaluated in the breeding nursery. In 1994 they were transplanted at a distance of 5 × 1,5 m in the breeding plantation. Another aim of the programme, along with obtaining rootstocks for sweet and sour cherry cultivars of a weak growth rate, was the establishment of sour- and sweet cherry cultivars bearing fruits of a good sensory profile, having a moderate to weak growth rate, compact crowns, self-fertile, resistant to biotic and abiotic stress factors.

‘Schattenmorelle’ cultivar was included in the remote hybridization programme mainly because of its good resistance to winter frosts and recurrent spring frosts. It is late flowering and it shows high and regular fertility. The fruits have very good technological qualities. The cultivar is self-fertile. The trees are moderate in growth and have a very good ecological plasticity.

The major reason for including the cultivar ‘Polevka’ in the hybridization plan, was the weak growth rate of the trees combined with the drooping (‘weeping’) crown habit. The cultivar also shows complex high resistance to biotic and abiotic factors. The form ‘RannaZhukovskaya’ is spontaneous bud mutation of the Russian cultivar ‘Zhukovskaya’, found in the collection of the Fruit-Growing Institute in Plovdiv in 1989. Fruits possess good qualities, they are of the Morello type. Trees have good and regular fertility. The cultivar shows high frost resistance and a complex resistance to biotic and abiotic stress factors. ‘Erdinagygyümölcső’ cultivar is of the Morello type, with very good fruit qualities. It is of interest to breeding because of its hybrid origin and good crossability with sweet cherry cultivars. The cultivars ‘Stella’ and ‘Compact Stella’ are included in the programme as donors of self-fertility and ‘Compact Stella’ and ‘Compact Van’ – as donors of a compact habit.

The experiments carried out by a number of authors, as well as the results of our previous studies showed that much more hybrids are obtained from the combination sour cherry × sweet cherry than from the reciprocal one. Our observations confirmed that the hybrid success of sour cherry × sweet cherry grown in the breeding nursery, is significantly higher compared to the almost zero survival rate of the hybrids of sweet cherry × sour cherry. In principal, much less fruits are born from the latter combination, and, much less hybrid stones, respectively, their germination being worse and the few hybrid plants obtained most often die as early as the first vegetation in the breeding nursery.

The best results concerning the number of the obtained hybrids, which survived in the breeding nursery and were grown in the breeding plantation, were obtained from the combination ‘RannaZhukovskaya’ × ‘Stella’, from which 84 hybrid plants were grown until reaching fruit bearing age (Tabl. 1). Among them there was neither hybrid plants with good enough sensory characteristics of the fruits, nor trees of satisfactory weak growth rate, complying with the requirements to modern rootstocks for sweet cherry cultivars. The parental combinations ‘Schattenmorelle’ × ‘Compact Stella’ and ‘Polevka’ × ‘Stella’ did not produce good results of the desired quality and quantity, too. The combination ‘Erdinagygyümölcső’ × ‘Stella’ gave hybrid plants, which died at an early stage and could not reach the fruit-bearing age. From the parental combination ‘Polevka’ × ‘Compact

Van', producing the second largest population, 31 hybrids were grown. After observations, evaluation and selection, it was established that their fruits are of an unsatisfactory sensory profile. By deliberately neglecting the necessary technological cultivation cares, we created conditions for assessing the sustainability of the population to biotic and abiotic stress factors. Under those conditions, after many-year observations, two hybrids No. 20-181 and No. 20-192 were selected for their weak growth rate, pronounced drought resistance and pest and disease resistance. At present the two seedlings are 24 years old trees with very good vitality. During the long period of observations, no symptoms of diseases or pest injuries were detected. Under *in vitro* conditions they show very good and easy propagation capacities. In the first-year nursery they reach optimal thickness for grafting and they have a higher survival percentage of grafted buds compared to the other sweet and sour cherry cultivars. Studies on both hybrids as sweet and sour cherry rootstocks with a weak growth rate are still going on in the first- and second-year nursery and in the plantation for rootstock production by vertical layers.

Studies will be carried out in fruiting plantations for establishing the growth habits of the trees of the different sweet and sour cherry cultivars grafted on the new rootstocks.

The source tree of hybrid No. 20-181 has a weak growth rate and reaches the maximal height of 1,5 m. The stem is upright, with a smooth grey bark. There are no stem and root suckers.

The source tree of hybrid No. 20-192 has a weaker growth rate compared to hybrid No. 20-181 and reaches the maximum height of 1,3 m. The stem is upright, with a smooth light-brown bark. The crown is loose, scattered, with drooping branches at the lower part, the shape and habit being probably inherited from the mother parent, which shows the same characteristics of the crown. There are no stem and root suckers.

Conclusions

Hybridization between sweet and sour cherry species is easy to perform in practice, despite the double difference in chromosome numbers ($2n=4x=32$ in *P. cerasus* and $2n=2x=16$ in *P. avium*). The higher degree of lethality of the hybrids between sweet and sour cherry is probably due to the larger number of hybrids containing 24 chromosomes in the somatic cell. It has been established that exactly those hybrids of *Prunus* genus have the lowest survival rate. It is known that the reciprocal hybrids *P. cerasus* × *P. avium*, which contain 32 chromosomes in the somatic cell, have lower lethality and respectively, much better survival rate, and a considerable number of them attain fruit-bearing age, most of them being fertile.

The parental combination *P. Cerasus* ('Polevka') × *P. Avium* ('Compact Van') is a successful result of the breeding activities for obtaining weakly growing rootstocks for sweet and sour cherry cultivars.

Table 1. Parental combinations and number of hybrids attaining fruit-bearing age

Parental combinations	Number of survived hybrids
<i>P. cerasus</i> 'Schattenmorelle' × <i>P. avium</i> 'Compact Stella'	1
<i>P. cerasus</i> 'RannaZhukovskaya' × <i>P. avium</i> 'Stella'	84
<i>P. cerasus</i> × (<i>P. cerasus</i> × <i>P. avium</i>) M-15 × <i>P. avium</i> 'Stella'	0
<i>P. cerasus</i> 'Polevka' × <i>P. avium</i> 'Stella'	8
<i>P. cerasus</i> 'Polevka' × <i>P. avium</i> 'Compact Van'	31
Total	124

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EVALUATION ON SOME NEW INTRODUCED SUMMER PEAR CULTIVARS IN PRODUCTION REGIONS IN R. MACEDONIA

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Abstract

In the paper is presented evaluation of biological and pomological characteristics of some new introduced summer pear cultivars. Six pear cultivars with different ripening time (Etruska, Turandot, Norma, Karmen, Toska and Harrow Delight) were evaluated during the research period. The cultivars Trapesiza, Cosia and Butira Precoce Moretini were used as control. In March 2009 experimental orchards were established in two agorecologically different regions: Veles and Valandovo. Wild pear (*Pirus communis* L) was used as a rootstock in Veles location, and quince MA in Valandovo. The following parameters were investigated: blossom time, ripening time, growth of the trees, yield, pomological characteristic of the fruits and content of the soluble solid matters. Sensory evaluations on organoleptic characteristics of the fruits were done also.

Some of new introduced summer pear varieties are characterized with a good production attributes. The fruits have good appearance and taste and they are attractive for consumers. The variety Trapesiza, Etrusca and Norma can be recommend for cultivation in agro ecologically conditions of Republica Macedonia.

Key words: pear variety, growth, productivity, fruit attractiveness

Introduction

Pear is a high-quality fruit, highly valued and required by the consumers wich caused a constant growth worldwide. In last 30 years the production increased for more than 2.5 times. In Republic of Macedonia in the last few years due the relatively low prices of the traditional fruits (apple) increased demand and relatively high prices, the areas and the production of pear started to grow. In the modern pear production, the choice of cultivar is a one of the key factor for profitable production. The world's fruit breeding centers constantly offering new cultivars whith better production characteristics than old cultivars.

Quality of the fruit is one of the most important goals in pear breeding programs over the world. The quality is particularly important for early summer cultivars. New cultivars should have a medium to large fruit. Fruit length should be greater than 7 cm and a width greater than 6 cm (Bell et al., 1996). The main aim of European breeding programs is high quality, size and attractiveness of the fruit. American breeding programs also share a number of common purposes such as fruit quality and diseases and pest resistance

(Milatovic 2009). The adaptation to the ecological conditions including the pest and diseases resistance is the main goal of selection of new pear cultivars (Bellini 2002).

Extension of the harvest season is very important aim of many breeding programs. The release of early summer cultivars looks very promising, though expected to occupy a limited place in the market (Bagnara and Laghi, 1997). There is a general need of new early ripening desert pear cultivars that ripen before the cv. Bartlet with equal or similar fruit characteristics (Bellini and Nin, 2002). Although numerous pear varietal novelties have been released in the last few decades, only very few of them have the potentiality to replace the standard cultivars which are very well known to the consumers and producers as well (Bellini and Nin, 2002). The existing pear assortment worldwide is becoming too conservative thanks to stable consumer habit regarding the pear characteristics (Gliha, 1997). Main pear characteristics important for customers are fruit shape, size, skin color, taste and flesh texture, whereas growers are interested in resistance to unfavorable conditions, resistance to diseases and pests, early yielding and productivity (Lace and Lacis 2015). Pear in Republic of Macedonia is mainly used for fresh consumption, where the size, appearance and taste are dominant quality indicators. Resistance on biotic and abiotic factors (diseases and pests, low and high temperatures, winds etc.) are also very important (Ristevski, 2004). The choice of suitable cultivar with late ripening time is quite wide, instead of those which ripen during the summer period (Kiprijanovski, 2006).

Therefore, the aim of this research is evaluation of some biological and economical characteristics of six new introduced pear varieties, which belongs to the group of extremely early and early summer cultivars (according to the ripening time). Based on this research some of them could be propose for introduction in agroecological condition of Republic of Macedonia.

Materials and methods

The research was conducted at two locations: Valandovo – southern part and district Veles - central part of R. Macedonia. All cultivars at the trial orchard Valandovo, Trapesiza, Etruska, Turandot, Norma, Karmen, Precoce Morettini, Harrow Delight, Cosia and Tosca was planted in the spring 2009 andrafted on quince MA as rootstock. Planting distance in orchard was 4x1.5 m (1666 trees/ha). Tress were trained by slender spindle system. Experimental plot Veles was planted with cultivars Etruska, Turandot, Norma, Karmen, Harrow Delight and Tosca in autumn 2009, with wild pear (*Prunus communis* L) as rootstock. Planting distance was 4 x2.5 m (1000 trees/ha). Trees were trained by spindle bush system. In both experimental orchards standardized agrotechnical and pomotechnical measures were applied with drip irrigation system.

Blooming and ripening time have been observed. Vegetative growth of the trees has been followed through measurement of the trunk diameter. The productivity of the cultivars was determined by the number of the fruits per tree, yield per tree and estimation of yield hectar. The fruits for analysis were harvested at commercial maturity stage. The dimension and weight of fruits were measured. Content of soluble solid matters was deined by refractometer. Sensory evaluation (taste and external appearance) was conducted using 5-point rating system (mark 5 – excellent rating).

The statistical analysis of the results was conducted by analysis of variance (ANOVA) and LSD test using SPSS version 11.0

Results and discussion

Vegetative growth

The rootstock has influence to the diameter of the trees and TCSA (Table 1) (Webster 2001). The trees grafted on seedlings have higher diameter of the trunk which is common for all cultivars. The cultivars Harrow Delight and Etrusca, have the largest (72.35 mm and 68.03 mm), while the cultivar Turandot has the smallest trunk diameter. The cultivars Karmen and Trapesiza grafted on quince have statistically higher trunk diameter (67.67 mm and 64.24 mm) compared to the other cultivars on the same rootstock except in comparison with cv. Norma. Cultivar Turandot shows the lowest value for these parameters with statistical significant differences in comparison with all cultivars.

Table 1. Trunk diameter of the evaluated cultivars, mm

Cultivar	Diameter of the trunk							
	Location, Valandovo				Location, Veles			
	2013	2014	2015	Growth (2013-2015)	2013	2014	2015	Growth (2013-2015)
Trapesiza	49.32 ^{bc}	54.79 ^{bc}	64.24 ^{ab}	14.92 ^a	-	-	-	-
Etruska	48.22 ^{bcd}	52.91 ^c	55.45 ^d	7.23 ^{ef}	42.63 ^{bc}	54.52 ^{bc}	68.03 ^{ab}	25.40 ^a
Turandot	41.42 ^e	44.93 ^e	47.10 ^e	5.68 ^f	38.23 ^c	48.62 ^c	55.23 ^c	17.00 ^b
Cosia	43.20 ^{de}	46.21 ^{de}	54.62 ^d	11.42 ^{bc}	-	-	-	-
Norma	52.29 ^{ab}	57.82 ^{ab}	62.17 ^{abc}	9.88 ^{cd}	48.53 ^{ab}	56.52 ^{ab}	65.93 ^b	17.40 ^b
Karmen	56.60 ^a	62.9 ^a	67.67 ^a	11.07 ^{bc}	46.52 ^{ab}	58.62 ^{ab}	64.35 ^b	17.83 ^b
Precoce Morettini	50.39 ^{bc}	55.22 ^{bc}	58.55 ^{cd}	8.16 ^{de}	-	-	-	-
Harrow Delight	47.65 ^{cd}	53.50 ^{bc}	60.2 ^{bcd}	12.53 ^{ab}	51.3 ^a	63.21 ^a	72.35 ^a	27.05 ^a
Tosca	46.11 ^d	51.15 ^{cd}	58.11 ^{cd}	12.00 ^{bc}	50.3 ^a	56.87 ^{ab}	67.97 ^{ab}	17.67 ^b

Popov (1983) reported that the cultivar Trapesiza has a strong growth, with big broad pyramidal crown. Rivalta (2002) presented that the cultivar Turandot has intermediated vigor, upright growth habit, while the cultivar Karmen there is intermediate-high vigor, upright growth habit. According Gliha (1997) the cultivars Etrusca and Precoce Moretini have a vigor growth of the trees, and the cultivar Harrow delight has the trees with intermediate vigor.

Phenology

The pear varieties which blossom earlier have higher damage risk from the late spring frosts (Kiprijanovski 2002). Higher early spring temperature in 2014 caused early blooming of the evaluated cultivars in both locations compared with 2015 (Table 2). At Valandovo all cultivars bloomed and harvested earlier 2 to 5 days due the warmer climate. Cultivar Norma bloom later 2 to 4 days and Harrow Delight 1 day later than standard cultivar Williams. The other cultivars bloom earlier 2-4 days from Williams. The earliest bloomed cultivars Trapesiza, Turandot, Etrusca and Tosca. The cultivars are characterized by different ripening time and according to the classification of Gliha (1997) belong to the group of extremely early (Trapesiza, Etrusca), early (Turandot, Cosia, Norma, Precoce Moretini) and early intermediate cultivars (Karmen, Harrow Delight, Tosca).

SECTION 7. FRUIT GROWING

Table 2. Blossom and harvest time of the pear varieties (2014-2015)

Cultivar	Blossom								Ripening			
	Valandovo				Veles				Valandovo		Veles	
	2014		2015		2014		2015		2014	2015	2014	2015
	Beg.	End	Beg.	End	Beg.	End	Beg.	End				
Williams	24.03	4.04	3.04	12.04	26.03	6.04	4.04	13.04	26.08	27.08	29.08	30.08
Trapesiza	20.03	31.03	30.03	10.04	-	-	-	-	22.06	25.6	-	-
Etruska	20.03	3.04	31.03	11.04	22.03	4.04	1.04	13.04	2.07	3.07	5.07	7.07
Turandot	20.03	2.04	30.03	9.04	23.03	5.04	31.03	11.04	14.07	15.07	17.07	18.07
Cosia	20.03	4.04	29.03	12.04					17.07	18.07		
Norma	26.03	6.04	6.04	15.04	29.03	10.04	7.04	16.04	21.07	24.07	23.07	26.07
Karmen	22.03	2.04	1.04	11.04	25.03	4.04	3.04	13.04	1.08	4.08	6.08	10.08
Precoce Morettini	24.03	5.04	1.04	12.04					25.07	27.07	-	-
Harrow Delight	25.03	5.04	3.04	12.04	27.03	8.04	5.04	15.04	7.08	9.08	9.08	10.08
Tosca	21.03	2.04	29.03	10.04	23.03	31.03	31.03	13.04	7.08	10.08	12.08	14.08

Rivalta (2002) reported that the cultivar Turandot characterized with very early blooming time, 5 to 6 days before Williams, and harvest time is first decade of July, 30-35 days before Williams. The cultivar Norma characterized with late blooming time, like Williams, harvest time is mid July, 25-30 days before Williams. The cultivar Karmen characterize with intermediate to late blooming time, longer than Norma. Harvest time is last ten days of July (15-20 days Before Williams). The early ripening cultivar Tosca, in ecological condition of Emilia -Romagna ripens at mid July (20-25) days before Williams (Rivalta, 2002). According Popov (1983) the cultivar Trapesiza characterize with early blooming and harvest time is at the end of June to beginning of July. Bergamaschi (2004) stated that cultivar Etruska ripen 21-49 days before Williams, while cultivar Tosca ripen 13-27 days before Williams.

Number of fruits and yield

Pear trees at experimental orchard in Veles had less fruits per tree, due to younger and more vigorous trees (Table 3). The most productive cultivars at orchard in Valandovo was Trapesiza with 100,1 furits/tree followed by cv. Cosia (77.3) and Etruska (55.9) with statistically significantly deferring between cultivars. Less productivity were registered at cv. Karmen (22.9) and cv. Norma (25.4) statistically significantly different from other evaluated cultivars. The most productive cultivar in Veles orchard was Etruska (57.4) followed by Tosca (37.6). The least number of fruit have registered at cultivars Karmen (21.8) and Turandot.(28.5).

SECTION 7. FRUIT GROWING

Table 3. The number of fruits and yield per tree

Cultivar	Number of fruits per tree						Yield per tree, kg					
	Valandovo			Veles			Valandovo			Veles		
	2014	2015	M	2014	2015	M	2014	2015	M	2014	2015	M
Trapesiza	115.73 ^a	84.5 ^a	10.1 ^a	-	-	-	9.98 ^d	6.46 ^b	8.22 ^a	-	-	-
Etruska	58.5 ^c	53.3 ^b	55.9 ^c	51.4	57.4	54.4 ^a	6.32 ^c	6.98 ^b	6.65 ^{bc}	5.93	7.34	6.62 ^b
Turandot	47.9 ^d	28.1 ^{de}	38.0 ^{ef}	27.4	29.6	28.5 ^{bc}	4.76 ^{cd}	3.66 ^c	4.21 ^d	3.93	4.45	4.19 ^c
Cosia	70.2 ^b	77.3 ^a	73.7 ^b	-	-	-	5.79 ^c	6.36 ^b	6.08 ^c	-	-	-
Norma	26.8 ^f	24.0 ^e	25.4 ^g	27.7	34.4	31.1 ^{bc}	5.09 ^{cd}	4.95 ^{bc}	5.02 ^d	5.44	8.19	6.80 ^b
Karmen	21.5 ^{fg}	24.3 ^e	22.9 ^g	18.7	24.9	21.8 ^c	3.88 ^{de}	4.94 ^{bc}	4.41 ^d	4.14	6.13	5.14 ^c
Precoce Morettini	40.6 ^{de}	42.0 ^c	41.3 ^{de}	-	-	-	8.17 ^b	6.82 ^b	7.50 ^{ab}	-	-	-
Harrow Delight	31.5 ^{ef}	35.6 ^{cd}	33.6 ^f	27.9	38.7	33.3 ^b	3.95 ^d	5.35 ^{bc}	4.65 ^d	5.27	8.41	6.84 ^{ab}
Tosca	15.1 ^g	79.7 ^a	47.4 ^d	27.9	47.3	37.6 ^b	2.76 ^e	11.75 ^a	7.26 ^{ab}	5.27	10.28	7.78 ^a

Among tested cultivars in Valandovo, cv. Trapesiza have the highest average yield (8.22 kg/tree), followed by cultivar Precoce Morettini (7.50 kg/tree), Tosca (7.22 kg/tree), Etruska (6.65 kg/tree) and Cosia (6.08 kg/tree). At the orchard in Veles the highest yield has cv. Tosca (7.78 kg/tree) followed by Harrow Delight (6.84 kg/tree) and Norma (6.80 kg/tree). The lowest average yield has cv. Turandot (4.19 kg/tree) and Karmen (5.14 kg/tree). Popov (1983), emphasizes that cv. Trapesiza is characterized with very good and regular yielding. According Gliha (1997), the cultivars Harrow Delight and Etruska are very productive, with regular bearing. Rivalta (2002) uncertain that the cultivars Turandot and Karmen characterize with medium-high yield, while Norma describes as medium productive cultivar.

Pomological and quality characteristics

Main characters important for customers are appearance (fruit shape, size, skin color), taste and flesh texture, (Lace 2015). Analyzing the data from pomological characteristics it can pointed that the average values for various parameters are different at the certain cultivars (Table 4). There were no significant differences in fruit weight between years; however, there were significant differences between cultivars.

Table 4. Pomological characteristics of the fruits (average 2014-2015)

Cultivar	Valandovo			Veles		
	Weight, g	Height, mm	Width, mm	Weight, g	Height, mm	Width, mm
Trapesiza	76.3 ^f	73.5 ^e	49.7 ^e	-	-	-
Etruska	117.6 ^e	78.7 ^{cd}	57.0 ^d	121.7	72.9	60.7
Turandot	130.2 ^d	89.6 ^b	59.1 ^{cd}	147.0	110.6	62.9
Cosia	82.5 ^f	75.6 ^d	56.4 ^d			
Norma	189.9 ^a	104.2 ^a	60.8 ^c	217.3	109.6	71.8
Karmen	194.1 ^a	105.1 ^a	69.6 ^a	233.8	114.2	74.5
Precoce Morettini	182.4 ^{ab}	83.3 ^c	70.8 ^a	-	-	-
Harrow Delight	152.1 ^c	70.2 ^e	64.3 ^b	153.8	72.4	67.6
Tosca	170.5 ^b	69.1 ^e	65.2 ^b	203.0	81.8	73.1

The cultivar Trapeziza has the lowest weight of fruit (76.3 g), followed by Cosia (82.5 g). Cultivars which are characterized with larger fruits are Karmen (194.1 g in Valandovo and 233.8 g in Veles), Norma (189.9 g in Valandovo and 217.3 g in Veles). The cultivar Etrusca and Turandot have fairly large fruit considering the time of ripening.

The cultivars Turandot, Norma and Karmen are characterized with good fruit organoleptic characteristics (Table 5), and good overall resistance to handling and storage (Rivalta, 2002). Syrgiannidis (2010) reported that the fruits from cultivar Etruska have weight of 137 g, length 90 mm, width 55 mm, total soluble solids 12.01 and titratable acidity of 0.24 g/100 ml. Bergamaschi (2004) conclude that the cultivar Etrusca have productivity 3-5 points, fruits with weight 85 -120 g, 10.2 -13.2 °Brix, appearance 3-4 and taste 2-4 points depend of district of production. The cv. Turandot has a productivity of 3-5 points, fruit with weight 150-190 g, appearance 3-4, and tastes 2-3 points. The cultivar Norma evaluated with 2-5 points depend of production region, according appearance and taste evaluate with 3-4 points. Gliha (1997) carried out that the cultivar Cosia because of small fruits is not competitive to the market on cultivars like Precoce Moretini, Norma and Precoce de Trevoux which ripen a few days late and have more quality fruits.

Table 5. Quality characteristics of the fruits

Cultivar	TSS, % Brix	Total acids, %	Appearance (0-5)	Taste (0-5)
Trapezica	12.1	0.22	3.5	3.8
Etruska	11.0	0.18	3.0	3.5
Turandot	11.1	0.20	3.5	3.4
Cosia	11.2	0.26	2.3	2.7
Norma	11.4	0.30	4.2	3.6
Karmen	12.2	0.49	3.8	4.3
Precoce Moretini	12.6	0.24	3.5	4.0
Harrow Delight	11.9	0.25	3.0	3.0
Tosca	10.9	0.31	4.0	2.5

Iglesias (2005) carried out that the fruits from cultivar Etrusca have 10.9 % TSS, total acidity 1.5 g/l, and width 55.9 mm. The fruit from cultivar Tosca content 11.6 % TSS, total acidity 28.5 g/l, and width 58.1 mm.

Conclusion

The introduced pear cultivars have particular positive characteristics which are significant for the producers and the consumers. All the valuated varieties have good compatibility with quince as a rootstock. The rootstock have a big influence on the fruiting and grown of the trees. The trees on quince rootstock have lower vigorous, with higher productivity per tree in the early period of fructification. The introduced varieties allow expansion of the season of consumption of quality pear fruits. According to ripening time, positive production characteristics, sensor features and the attractiveness cultivars Trapeziza, Etruska and Norma cultivars can be recommended for cultivation in Republic of Macedonia. They could replace the old assortment of early and medium early summer cultivars.

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INFLUENCE OF THE VARIETIES ON THE OPTICAL PROPERTIES OF APPLE CHIPS PREPARED WITH OSMOTIC TREATMENT

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Abstract

The aim of this research is to examine the effect of the varieties on the qualitative characteristics of the apple chips obtained by applying osmotic drying as pretreatment. For this aim apple chips is prepared from five apple varieties: Idared, Mutsu, Jonagold, Granny Smith and Red Delicious. Optical properties are examined using a colorimeter Dr. Lange spectro-color. The samples were cut with a special knife with thickness 1 to 2 mm in the form of a disc with a diameter of 18 mm. Osmotic solutions were prepared from aqueous solution of sucrose with a concentration of 40%, 50% and 60%. After 60 min osmotic treatment, samples were dried in air-dryer at 100°C, for 120 min. The pieces of the apples treated with osmotic treatment have lower values of browning index compared with samples of conventionally dried apple pieces. This indicates that the samples with previous osmotic treatment retain the natural color and reduce the intensity of the enzymatic and non-enzymatic browning. With the increasing concentration of the osmotic solution we get reducing of the browning products. The absorbed sucrose molecules impede the path of transmission of oxygen to the polyphenol compounds. Thus, the degree of browning has decreased by increasing the temperature, concentration of the osmotic solution and by increasing time treatment. The best results showed the variety Idared while the worst results were obtained from the variety Red Delicious. These results were obtained because the variety Idared has the highest percentage absorbed sucrose molecules while the lowest percentage has the variety Red Delicious.

Key words: apple, osmotic treatment, optical properties.

Introduction

The osmotic dehydration can be defined as a process of simultaneously dehydration and sorption with immersion in osmotic solution. During that, in the biological material two contrary processes of mass transmission happen. This can happen because of the osmotic pressure which appears as a result of the difference in concentrations of the osmotic solution and the solution in the tissues in the biological material through the cell membrane of the tissues. The cell membrane is not selective, and that is why the other components of the biological material, such as: carbohydrates, organic acids, minerals and vitamins are removed together with the water. However, the diffusion of these components is

insignificant compared to the waste of water and absorption of dissolved particles from the osmotic solution. Still, they are very important for the final product quality. (Mastrantonio *et al.*, 2005).

Many researchers have showed that the quality (color, texture and rehydration capacity) of the products which have been air dried, freeze dried and vacuum dried improves with application of osmotic dehydration as a pre-treatment (Hawkes and Flik, 1978; Lerici *et al.*, 1985; Nsonzi and Ramaswamy, 1998). The osmotic pre-treatment stabilizes the color parameters of the food products and allows lower color losing with enzymatic browning because of the penetration of dissolved matters. (Liping, 1998).

The osmotic dehydration, except that is known for keeping the natural color, texture and nutritive value of the treated inputs, has other advantages too.

Materials and methods

Five apple varieties are used as material: Idared, Mutsu, Jonagold, Granny Smith and Red Delicious, bought in the local green market in Skopje and kept on temperature of -4°C . Sucrose is used for preparation of osmotic solution.

The examined apples are cut in circular shape with thickness of 1-2 mm and 18 mm diameter. The prepared samples are measured in analytical balance with $\pm 0,0001$ g readability and are immersed in osmotic solution prepared with dissolving of commercial sugar in distilled water. The ratio between the sample mass and the osmotic solution was 1:15. The change of color of the apple samples was monitored with Dr. Lange spectro-color colorimeter. The color was monitored at fresh, osmotic treated samples, as well as at samples dried in conventional dryer, in different time intervals, 15, 30, 45, 60, 90, 120 and 180 minutes. Before usage, the colorimeter is calibrated with white and black disk according to the manufacturer instructions. Three parameters are measured: L^* (brightness), a^* (greenishness) and b^* (yellowishness). With the help of this parameters, the index of browning is calculated using equations described by Maskan (2001):

$$BI = [100(x - 0,31)] / 0,17 \quad (1)$$

Where:

$$x = (a + 1,75L) / (5,645L + a - 3,012b)$$

Results and discussion

The color analysis of the products is measured with the help of the color parameters L^* , a^* and b^* . L is brightness of the sample with values from 0 for black and 100 for white. The negative values of the parameter a show presence of greenish color, while the positive values show presence of reddish color. The parameter b^* represents yellow color. In table 1 the experimental data for the three color parameters and the calculated values for the color (chroma), the change of color (E) and the browning index (BI) monitored for 15-180 minutes for variety Idared, at concentration of osmotic solution of 60%, are showed. As it can be noticed from the table 1, over time, the values of L^* parameter slightly decrease, which means that the browning of the product is happening more slowly. Contrary of the L^* parameters, the values of the other two color parameters a^* and b^* slightly increase. That is completely expected because over time the products start to turn red and turn yellow. The parameters L^* and a^* show the enzymatic browning, which is caused by polyphenol oxidase at moderate temperatures. The same happens with the color, the

change of color and the browning index which are calculated from the measured values of the three color parameters. The values of these quantities are also expectedly increasing, over time. The browning index (BI) gives the products brown color. The parameter b^* contributes the most for color changes (E). The same happens at the other examined apple varieties Mutsu, Jonagold, Granny Smith, Red Delicious.

Wais *et al.* (2005) followed the color parameters of osmotic dehydrated fruits of the variety Granny Smith in water sucrose solutions. According this investigation, the values of the L^* parameter of color were increasing, while the a^* and b^* color parameters were decreasing over time. Gobbi (2009) who worked with variety Golden Delicious reported that L^* color parameter is decreasing over time. The same was concluded by Chang *et al.* (2003) who have worked with green pumpkins. In the table 1 are data of the experimental values of the color parameters at variety Idared measured under different drying techniques and different osmotic solution concentration are given.

Table 1. The color parameters, change of color and index of browning at the variety Idared

Time.(min)	Color parameters					
	L	a	b	Color	Change of color	Index of browning
Fresh	62.71	-0.73	5.64	5.68	1.87	8.33
15	62.40	-0.63	6.12	8.28	3.58	9.32
30	61.18	-0.53	6.21	6.25	1.69	9.79
45	61.00	-0.46	6.86	6.90	2.45	11.07
60	61.29	-0.40	6.98	6.94	1.84	11.31
90	61.86	-0.38	7.24	7.29	3.91	11.69
120	60.66	-0.25	8.29	7.32	4.05	14.03
180	60.23	-0.12	11.62	7.97	3.97	20.77

As it can be seen from the tables, the parameter L (brightness) value is decreasing at osmotic, air and conventional drying, while at the parameter a-greenishness, value is increasing compared to values of the fresh apples. That shows that under these drying techniques, the enzymatic browning reactions stick out. The fruits of variety Jonagold are an exception at which, under conventional drying, the L values are higher than the L values at fresh samples. It is assumed that under conventional drying of fruit of this variety, dissolution of tiny quantity of pigment happens, while under osmotic dehydration bigger quantity of pigment flows to the osmotic solution. Unlike L parameter under all the drying techniques at all varieties, a and b parameters are increasing compared to the fresh samples. The differences of these parameters under osmotic dehydration are very small compared to the air and conventional drying. The highest values at a and b parameters (especially parameter b) can be seen at conventional drying because under this drying technique the pigments are dissolving (Maskan, 2001).

SECTION 7. FRUIT GROWING

Table 2. Color parameters at the different concentration of osmotic solution at the different techniques of drying

Variety	Idared			Mutsu			Jonagold			Granny Smith			Red Delicious		
Way of drying	L	a	b	L	a	b	L	a	b	L	a	b	L	a	b
Flesh	62.72	-0.73	5.64	63.07	-0.84	10.82	63.78	-0.28	8.02	63.60	-3.07	8.74	63.43	-1.25	10.56
Air	59.85	2.00	13.74	57.31	1.72	14.76	62.88	-0.24	13.11	60.76	-0.65	10.15	55.43	0.98	16.17
Conventional	56.76	11.15	20.83	61.97	9.21	22.45	68.86	6.48	23.53	54.95	8.72	19.05	58.41	10.53	22.19
40%	63.84	-0.64	12.42	64.44	-0.26	14.29	58.59	0.01	11.43	61.17	-1.44	10.61	58.74	-1.12	10.17
50%	61.17	-0.16	13.08	55.80	0.06	9.38	56.37	-0.60	8.75	62.54	-1.26	8.76	56.01	0.71	14.79
60%	60.23	-0.61	7.94	55.29	-0.25	11.65	56.57	-0.19	9.98	63.01	-1.73	11.26	55.61	-0.46	11.38
Combine d-40%	76.64	2.04	23.27	70.83	2.81	21.52	67.27	5.13	19.95	64.65	4.28	21.36	63.47	6.85	22.28
Combine d-50%	71.98	3.29	19.61	70.34	2.39	20.70	68.08	1.89	18.73	64.70	3.87	19.74	70.41	3.97	26.75
Combine d- 60%	65.53	198	17.62	70.85	4.57	23.53	68.44	2.88	21.60	68.11	3.68	19.91	71.00	6.07	24.06

In table 2 the color parameters are showed at products made with conventional drying, with and without osmotic treatment. The apples which are submitted to osmotic treatment have the closest color parameter values with the values of the fresh samples. Under osmotic treatment the L parameter values are higher, and values at a and b parameter are lower. Under osmotic dehydration the interaction between the substratum and the enzyme polyphenol oxidase, which is responsible for enzyme browning of the apples, is disrupted because of penetration of the sucrose molecules in the plant tissue. Quileset *et al.* (2005) analyzed the activity of the enzyme polyphenol oxidase on fresh and osmotic treated fruits at variety Granny Smith in water solutions of sucrose. The activity of the enzyme polyphenol oxidase on osmotic dried Granny Smith apples was lower compared to the fresh apples because of the penetration of the sucrose molecules during the osmotic treatment.

The osmotic dehydration decreases the intensity of Maillard reactions (non-enzymatic browning). This is due to the flowing of the amino acids and carbohydrates to the osmotic solution. The penetrated sucrose molecules in the plant tissue extrude the inner gases which have protective role for pigments. Under conventional drying, the pigments in the apples dissolve. The presence of sucrose molecules on the surface of the samples is an obstacle for the contact with oxygen. In this way, the oxidative reactions in the osmotic dehydrated samples are decreasing (Mauro *et al.* 2005).

From the color parameters values from the experiment, the browning index for all the apple varieties is calculated according to the equation 1. With its help, the browning of the products is followed. The results for the browning index are shown in picture 1. As it can be seen in picture 1, the osmotic dehydrated samples have the best result. The differences between the dried samples with and without osmotic treatment are obvious. The apples treated with osmotic treatment have lower browning index values compared to the apple samples which were dried conventionally. That shows that the samples which were previously treated osmotically keep their natural color and have lower intensity of enzymatic and non-enzymatic browning.

Jokis *et al.* (2009) dried fruits of variety Florina conventionally with and without osmotic treatment. They ascertained that the osmotic pre-treatment decreases the total change of color (ΔE) for 47% compared to the untreated samples.

From the picture 1 it can be seen that with increase of the osmotic solution concentration the products browning is decreasing. We get these results because with increase of the osmotic solution concentration, the absorption of sucrose molecules is increasing, which contributes to decreased enzyme-substratum reaction between the polyphenol oxidase enzyme and polyphenol compounds which are present in the apples. The absorbed sucrose molecules obstruct the way of oxygen transport to the polyphenol compounds. Chang et al. (2003) examined the impact of the concentration, temperature and duration of the osmosis to the level of browning of the green tissues. According to them, the level of browning decreased with increasing of the temperature, the concentration of the osmotic solution and the duration of the treatment.

From the analyzed apple varieties from picture 1, the variety Idared has the best results, while the Red Delicious variety has the worst results. We get these results because the variety Idared has the highest percent of absorbed sucrose molecules, while the fruits of variety Red Delicious has the lowest. The absorption of bigger quantity of solid substances contributes to the decreased activity of the polyphenol oxidase enzyme. Also, it contributes to decreased oxidative reactions.

Conclusions

With increased duration of the osmotic treatment, the browning index values of the products increase, while with increased concentration of the osmotic solution, the browning index values are decreasing. The apples previously treated with osmotic treatment keep the natural color and decrease the intensity of the enzymatic and non-enzymatic browning. From all analyzed apple varieties, the variety Idared has the best results, while the variety Red Delicious has the worst results.

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**INFLUENCE OF USING MULCH AND HERBICIDE ON VEGETATIVE
PARAMETERS OF TWO APPLE CULTIVARS**Cikaqi B.^{1*}, Lepaja L.², Lepaja K.², Zajmi A.³¹Ministry of Agriculture Forestry and Rural Development, Kosovo,²Agriculture University of Tirana, Koder-Kamez, Tirana, Albania.³University of Prishtina, Faculty of Agriculture and Veterinary, Kosovo.

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Abstract

The use of mulch in orchard is increasing in worldwide. The main reasons for the use of mulch are: conservation of moisture in the soil, prevent weeds, add organic matter to soils thereby increasing their mineral content etc. In this research paper we have presented one year result. The aim of the research was to determine the best way of maintaining the apple orchard in order. We used three type of treatments, the first control, the second we used mulch (wood chips) at thickness of 10 cm and third we used total herbicide. The experiment was conducted in Kosovo in 5 ha of apple orchard, two cultivars are: Gold Delicious clone Rainders and Braeburn on M9 root stock. The experiment was set up according to the randomized complete block design (RCBD) methods in four replications, one replication were five trees. Using ANOVA we found significant changes to leaf surface, for cultivar and treatments differences were at level ($P < 0.01$) for their combinations at level ($P < 0.05$) while changes were not significant for diameter of trunk first and second measurement even if mulching had a positive effect on all parameter values.

Key words: Braeburn, G. Delicious-Rainders, M9, mulch, herbicide.

Introduction

Production of apple considered (*Malus* sp) that is of particular importance for the economy of Kosovo. Until now about 2024 ha are planted with apple. Mulch is a powerful strategy for management of unwanted drugs and could be contributing to a better land use management, if the adequate natural material is used. After planting is stabilised, unwanted herbs could be controlled by using thick layers of mulch. Organic mulch is used usually in surrounding area of a vine fruit plant and through the whole line.

M9 root system is developing in shallow surface of the ground, therefore is required enforcement or supporting concrete pillars, they could be combined through horizontal wire, especially in regions with frequent appearance of winds. M9 rootstock does not have sufficient compatibility with other cultivars, especially with red Delicious, where bellow the root stock is appearing fattening hump. Above M9 rootstock should be implanted apple cultivars with medium and high production rate (Zajmi, 1997).

According to Lepaja, (2011) in his research showed that MM106 reached greater leaf surface then M9 rootstock.

Mulch prevents the appearance of unwanted herbs, reduces evaporation, increase the organic content of the land, and increase the feeding element of the land etc. but he also performs another important function, this time directly on growing activities of the plant(Downer, 2009).

Increase of the organic substances in an apple orchard showed increase of flowering (Autio *et al.*, 1991).Diversification of organic substances are used as mulch to prevent appearance of unwanted herbs and to improve the land fertility (Cogliastro *et al.*, 1993; Pinamonti, 1998; Arthur and Wang, 1999; Lu *et al.*, 1999).

Mulch showed positive results by increasing values in many vegetarian parameters and production then other varieties without mulch (Lepaja *et al.*, 2014;Lepaja *et al.*, 2015).

The objective of this study was to determine the impact of mulch (wood chips) and herbicide on vegetative parameters in apple orchard in Kosovo.

Materials and methods

Our investigations were conducted during the year of 2012 (second year of orchard) in commercial apple orchard with an area of 5.0 ha located in region of Drenas. In this research paper we have worked with two apple cultivars (Braeburn and Golden Delicious clone Rainders). Planting distance for both cultivars (Braeburn and Golden Delicious clone Rainders) on rootstock M9 was 3.20 x 1.60 m. The Orchard had a drip irrigation water system. At random we have selected the fruit trees that were included in our investigations. The experiment was set up according to the random complete block design (RCBD) methods in four replications. The size of experimental was 5 trees for each treatment. Experimental plots are marked with corresponding adhesive bandage for each treatment and repetition. In one parcel are taken five plants for processing for each treatment (with wood pieces (mulch), the second with herbicide; and third control) that means there are included 120 apple tree from two apple cultivars.

The version with mulch was performed in 10cm thickness. Mulching material was placed in a row of a width of70 cm. For each cultivar are taken 4 repetitions.

Use of “Glikosav” herbicide, 50 ml per 10 litre of water, with the hand pump of 10 litre, is used on trees included in research 70 cm wide in the row.

Control area without mechanical intervention for any cultivar in four repetition (4 field with cultivar). All experimental fields are visibly marked with sticks and rope.

Tree diameter is measured with calibrator, 10 cm above the graft site, whose values are expressed in mm. Measurements were performed at the beginning and at the end of the vegetation. The leaf surface. By the measurement of randomly selected 10 leaves from the plants included in the experiment is determined. Values are expressed in cm² / foliage. Measurements are presented in cm²(on November 2nd, 2012). Data from the measurements were analysed using ANOVA.

Our state has a moderate continental climate with a coastal impact which penetrates through the valley of the Driniibardhë moderating markedly continental climate elements (Lepaja *et al.*, 2014; 2015). In Kosovo average temperature multiyear (1951-1980) is 10.3 °C, that of vegetation 16.5 °C, the coldest month is January (-0.9 °C) while the hottest month is July with 20.1 °C. Regarding the annual rainfall is 744.8 mm, and during vegetation is 346.7 mm which shows the need to intervene with supplementary irrigation (Zajmi, 1996). Water shortages in the territory of Kosovo, especially during the vegetation period, need supplemental irrigation.

Result and discussion

During one year of our research for the impact of land maintenance in one apple orchard, respectively maintenance of land with mulch, herbicide and control for apple cultivars Braeburn and Golden Delicious, clone Rainders, in the agroecologic condition we come to the results presented in table below.

Table. 1. Medium values for measured parameters

Parameters		Diameter of trunk (mm) 1 st measurement	Diameter of trunk (mm) 2 nd measurement	Difference between two measurements (mm)	Leaf surface (cm ²)
Cultivars (A)	Treatments (B)				
Braeburn	Mulch	139.45 ^a	229.5 ^a	90.05 ^a	23.12 ^b
	Herbicide	132.97 ^a	217.03 ^b	84.06 ^{ab}	21.75 ^b
	Control	133.25 ^a	204.87 ^d	71.62 ^b	18.75 ^c
G. Delicious clone Rainders	Mulch	134.87 ^a	221.57 ^b	86.7 ^a	32.4 ^a
	Herbicide	136.48 ^a	213.65 ^c	77.17 ^b	33.23 ^a
	Control	140.78 ^a	215.95 ^c	75.17 ^b	23.73 ^b

Legend: Averages listed, in column, in subscript with different letters are most important $P < 0.05$

Average values of a tree plant of the researched cultivars showed different values in first measurement. Average values of the tree plant Braeburn were between 132.97-139.45 mm, while to Gold Delicious 134.87 – 140.78 mm (table 1.). Based on variance analyses, there were not significant average differences on tree plant diameters between cultivars for researched varieties.

When it comes to the tree diameter measurement for the second time in (mm) at the end of the vegetation it is realised different increase of tree diameter depending on the maintenance. Based on variance analyses (ANOVA) there were not significant average differences between the cultivars, but there were significant differences on maintenance in the row.

In second measurement higher diameter values showed Braeburn cultivar with mulch variety 229.5 mm but the weakest, also Braeburn in group control 204.87 mm. Important to mention is growing difference between treatments of Braeburn cultivar was about 25mm.

To the delicious cultivar are found significant differences between treatments maintain with mulch (221.6 mm) and treatments with herbicide 213.65 mm and control treatment (215.95 mm). There were not significant differences between treatments with herbicide and control. Worth to mention is that growing differences between treatments of Gold Delicious was 8mm.

The differences between two measurements, the first measurement and the second measurement conducted at the end of vegetation are presented in table 1; and based on analysis of variance (F test) have been found significant differences between cultivars, and between maintenance in the row. It is noticeable that tree diameter of two cultivars is lacking on control (71,62 mm in Braeburn cultivar and 75.17 mm in Gold Delicious clone Rainders). The highest increase of tree diameter at the end of vegetation, disregard maintenance in the row, showed Braeburn cultivar (81.91 mm) compare with Gold Delicious clone Rainders cultivar (79.68 mm). The differences in average were significant at level 0,01%. Concerning maintenance in the row disregard the cultivars are realised higher average growth of tree diameter on treatments with mulch (88.37 mm), then

treatment with herbicide (80.61 mm) and control treatment (73.34 mm). Significant differences in level of 0.01% are noticed between treatment with mulch and two other treatments, but between treatments with herbicide and control there were no significant differences. Based on this analyse we can conclude that on treatment with mulch the plant not only fought undesired herbs that are direct nutrition competitor but from this action the cultivated plant used better nutrition that reflected into tree diameter also.

The results for effect of soil maintenance in the row, in leaf surface after 5 months of experiment are (ANOVA) are presented in table 2.

Table. 2. Leaf surface (cm²) for two cultivars according to the maintenance in the row (ANOVA).

Treatments (A)		Cultivars (B)		Average (A)
		Braeburn	G. Delicious-Rainders	
Mulch		23.12	32.40	27.76
Herbicide		21.75	33.23	27.79
Control		18.77	23.73	21.25
Average (B)		21.22	29.79	
Factor		A	B	
LSD	1%	3.08	8.06	
	5%	2.20	4.39	

Based on (F-test) variance analyses are noticed significant differences of leaf surface between researched cultivars, as well as area maintenance in the row showed impact in leaf surface growth.

Based on analyses of individual area the highest leaf surface is discovered in Gold Delicious clone Rainders 29.79 cm² but the lowest Braeburn cultivar with 21.22 cm². Differences between this two cultivars are significant in 99% of the cases (LSD 0.01).

Based on LSD test the differences between treatments the control and two other treatments respectively mulch and herbicide treatments in level 0.01%, but between treatments with mulch and herbicide were not realised statistical discrepancies.

For the positive effect of mulch, better management of unwanted herbs speaks a number of authors like: Lučić *et al.*, 1996; Autio *et al.*, 1991; Cogliastro *et al.*, 1993; Pinamonti, 1998; Arthur and Wang, 1999; Lu *et al.*, 1999; Lepaja *et al.*, 2014; Lepaja *et al.*, 2015.

Conclusions

Based on our one-year research for the impact on ways for earth surface maintenance in the row in one apple orchards, respectively mulch maintenance of soil, with herbicide and control for two cultivars, Braeburn and Golden Delicious clone Rainder, carried out in agro ecologic conditions of Drenas, covering the area of 5.0 hectares, where is applied common technology for apple cultivation, we can come to this findings:

Mulch effects were noted by increase of all researched parameters compared with control treatments. In treatment with mulch there were no unwanted herbs, saving water and nutrition material for fruits in experiment (Lepaja *et al.*, 2014; 2015). The plant tree diameter in (mm) as in first measurement and in the second are reached high values in treatment with mulch, followed by herbicide and then control treatment. In the difference between two measurements the tree diameter increase is higher for Braeburn (mulch treatment) compare with Gold Delicious (mulch treatment). Based on (ANOVA) variance

analyses there are no significant differences in averages of the tree plant diameter for treatments maintaining in the row. Braeburn compares to Golden Delicious-Rainders showed the highest increase of the tree diameter by the end of vegetation process disregard maintaining in the row. The average of difference was significant in level of 0.01%. The leaf surface (cm²) higher values reached mulch treatment. The cultivar G. Delicious reached higher values in comparison with Braeburn cultivar. Significant differences are noticed between control treatment and other two treatments with mulch and with herbicide.

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**STUDYING THE CHANGE IN BIOCHEMICAL COMPOSITION OF
FRESH AND DRIED CHAENOMELES FRUITS**Georgiev D.^{1*}, Mihova-Chavdarova T.¹, Georgieva M.¹¹Research Institute of Mountain Stockbreeding and Agriculture, Troyan, Bulgaria

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Abstract

The study was conducted in 2014. For an object of the experiment was chosen three perspective forms of *Chaenomeles sp.* Lindl. distinguished by a number of significant vegetative and reproductive indicators. The chemical composition and degree of preserving of different components in fresh and dried fruits were studied. The following indicators were studied: dried matter according to Re%, total sugars, inverted sugar, sucrose, organic acids, ascorbic acid, anthocyanins, tanning substance and pectin. The three forms of *Chaenomeles sp.* were grown under nonirrigated conditions, with the application of necessary agrotechnical events. The fruits are distinguished by high content of ascorbic acid, which is in the range of 79.20 mg%-109.12 mg% in fresh fruits. The decrease of the same indicator in dried fruits was significant, which varied in the different forms from 2.9 times to 5.3 times. Significant differences were also observed in the values of other examined components. The aim of the study is to follow the change in the chemical composition between fresh and dried fruits.

Key words: *Chaenomeles sp.* Lindl, Fresh fruits, Dried fruits, Chemical composition

Introduction

Chaenomeles sp. Lindl. is a relatively unknown culture to agricultural farms. Plants could be used for decoration because of their beautiful flowering predominant in a few nuances. Fruits of studied genotypes have various forms - oblong, round, oval. They are rich in organic acids, ascorbic acid, tanning substances, pectin and other components. They are not suitable for fresh consumption, but they are perfectly suitable for drying and processing in syrups, jams and other products (Georgiev et al., 2013; Georgiev et al., 2013; Brashlyanova et al., 2014). There has recently been an increased interest towards this culture, namely because of the rich biochemical composition of its fruits. (Guo et al., 1997; Abbott, 1999; Dharmananda, 2005; Hong et al., 2007) The aim of this experiment is to study the biochemical composition of fresh fruits and the change of biochemical indicators after drying in a dryer with an alternative source of energy.

Materials and methods

The study was conducted in the Research Institute of Mountain Stockbreeding and Agriculture, Troyan. The research was conducted on three perspective genotypes of *Chaenomeles sp.* Lindl. Fruits were gathered from a collection plantation of the institute.

Drying was done in a dryer with an alternative source of energy. The chemical analysis of fruits was conducted in the chemical laboratory of the institute. The following indicators were studied: dried matter according to Re%, dry weight, total sugars, inverted sugar, sucrose, organic acids, ascorbic acid, anthocyanins, tanning substance and pectin. Daily temperature values and atmospheric humidity were reported periodically. Fruits were dried for two days at average daily temperature of 25 degrees and 45% atmospheric humidity (photo 1 and 2).



Photo 1. Fresh fruits from *Chaenomeles sp.* Lidl.



Photo 2. Dried fruits from Japanese quince

Results and discussion

Chemical analysis data are shown in Table 1. Dry matter content in fresh fruits was within the interval of 8.5%-10.5%. Total sugars were presented in small quantities. It was 3.35% for form 1, and considerably more for the other two forms, about 6%. Inverted sugar was the highest in form 2 - 5.2%. Sucrose was not reported for form 1. For form 3, it reached values up to 2.04%. Acid content in the fruits of Japanese quince is high. The values were almost the same for the three forms - approximately 2%.

The main indicator of the Japanese quince fruits, which distinguishes them from other fruit culture, is the high content of ascorbic acid. It was 79.20 mg% in form 1, in form 2 – 88.00 mg% and in form 3 it was much more – 109.12 mg% (Figure 1).

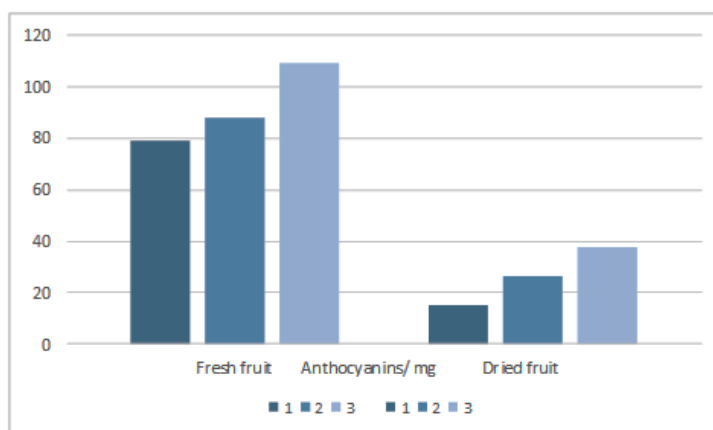


Figure 1. Vit.C (mg/%) of fresh and dried fruits of some forms of *Chaenomeles sp.*

Significantly higher were the values of tanning substances in studied genotypes, which were within the limits from 0.330% (form 1) to 0.400% (form 3).

The content of anthocyanins in analyzed fruits was the smallest, in comparison with other components. Their content was the smallest in form 1 - 1.13 mg% and about four times more than fruits from form 3 - 4.19 mg% (Figure 2).

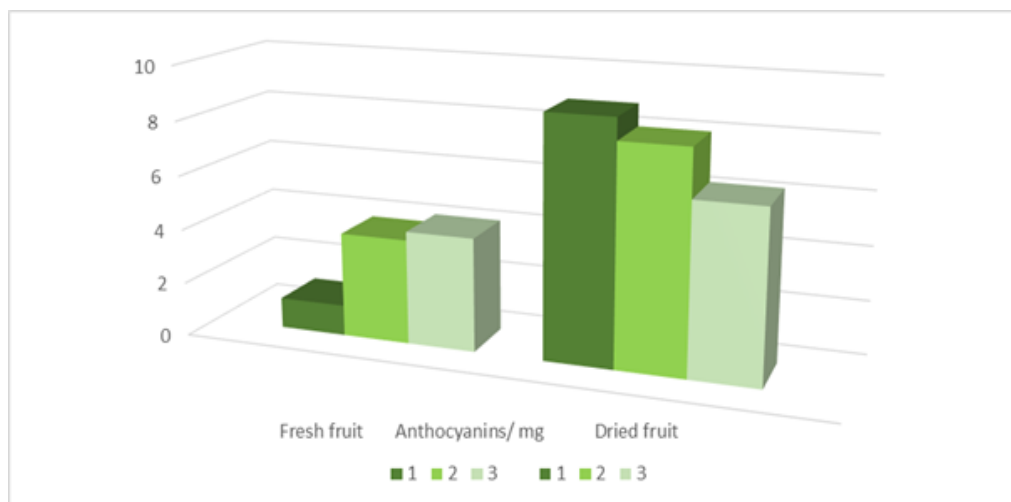


Figure 2. Anthocyanins (mg) of fresh and dried fruits of some forms of *Chaenomeles* sp. Lindl.

Pectin is the next indicator represented by high values, but in the present year they are lower than the usual for this culture. It was the highest in form 2 - 0.99%. Dry weight of fruits was 13% – 14%.

Table 1. Chemical composition of fresh and dried fruits of some forms of *Chaenomeles* sp. Lindl.

Genotype	DM (%)	Total sugars (%)	Inverted sugar (%)	Sucrose (%)	Organic Acids (%)	Tanning (sub. %)	Pectin (%)	Dry weight
Fresh fruit								
1	9	3,35	3,35	0	2,28	0,33	0,71	14,43
2	10,5	5,7	5,2	0,48	2,01	0,354	0,99	14
3	8,5	6	3,85	2,04	2,14	0,4	0,79	13,63
Dried fruit								
1		13,65	11,95	1,62	8,38	1,886	5,82	84,00
2		15,65	15,35	0,29	7,24	1,415	6,20	83,51
3		13,65	10,90	2,61	10,92	1,132	7,00	83,80

In dried fruits, dry weight was 83-84%, which means that drying was in standard norms (Table 1). Total sugars were 13 - 16%. Their increase compared to fresh fruits in different forms was from 2 to 4 times as much. It was the highest in form 1, but when the decrease was pre-calculated to dry units it was 2-3 times. Similarly, an increase was also noticed for values of inverted sugar. In form 3, it was 10.90% and reached up to 15.35% in form 2. Reduction towards dry units in first form was 3.5 times and 2.7 times for the second one.

There was a great variation of sucrose in analyzed genotypes, which varied within the interval - 0.29% (form 2) to 2.61% (form 3). The losses were respectively 13.5 and 7.7 times towards the dry units. There was a significant increase in acid content. In form 3 it was about 5 times more than in fresh fruits. The decrease was 1.9 times when it was recalculated towards dry units.

It is noticed the reduced content of ascorbic acid in dried fruits in comparison with fresh fruits. In form 1 the reduction was 5.3 times, in form 2 – 3.3 times and in form 3 was 2.9 times. To dry units the reduction was respectively: form 1 – 49 times; form 2 – 26.5 times; form 3 – 28.4 times. The increase of tanning substances in dried fruit was considerable. It was the highest in form 1 - 1.886%. The decrease was 1.6 times, when it was recalculated toward dry units. The content of other biologically active substance - anthocyanins, was considerably increased. The value in form 1 was 8.87 mg%, which was almost eight times than in fresh fruit. The decrease was insignificant towards dry units - 1.18 times. Pectin content reached the impressive 7% for form 3, which was 8.9 times more than in fresh fruit. The loss was insignificant - 1.1 times, when it was recalculated towards dry units.

Conclusions

Fruits of form 3 were distinguished by a higher content of biologically active substances. Studied forms are suitable for drying, as fruits of forms 1 and 3 have a greater advantage concerning the complete preserving of chemical composition.

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**IDENTIFICATION OF NEW EXPORT OPPORTUNITIES FOR FRUIT
AND VEGETABLE PRODUCERS**Muça (Dashi) E.^{1*}, Hodaj N.², Thoma L.³¹Lecturer, Faculty of Economy and Agribusiness, Agricultural University of Tirana²Lecturer, Faculty of Economy and Agribusiness, Agricultural University of Tirana³Lecturer, Faculty of Economy and Agribusiness, Agricultural University of Tirana

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Abstract

Fruits and Vegetables sector take a very important place not only as planted cultures in Albanian farms, but also in the consumption of Albanian population. Actually, in Albania exists an interior market in development and a non structured exporting market. Traditional rural areas in Albania are distinguished by a subsistence economy. Farms are extremely fragmentized and production units are too small. The average surface of Albanian farms is 1.26 ha. So as consequence there is no uniformity in the quantity and quality of the products. This situation makes it difficult to concentrate the produce for efficient exportation. In certain areas the vast majority of the population is not at all used to thinking in terms of commerce and barter trading. The main goal of the paper is to identify the export growth opportunities for fruit and vegetable producers. The instrument used for the survey was closed questionnaire. We interviewed the great part of fruit and vegetable farmers, who export their products. The majority of them lived in south west side of Albania. The information was processed in a simple data base in Microsoft Excel. As result we conclude that exist several problems that hamper exportation growth in Albania. Albanian producers usually are incapable to penetrate in foreign markets, because they are not part of consolidate marketing distribution channels.

Key words: exportation, fruit and vegetable producer, distribution channel

Introduction

The rapid urbanization that begun after the change of the communist regime and still continued in Albania, influence in the economic situation of the rural population. After 90 s' the situation in the sector, as in the whole Albanian Economy was very difficult. Reforms undertaken during the years 1991-1992 the Albanian agriculture led to a total anarchy (Civici et al., 2003). Also the majority of agricultural structures (as irrigation systems, greenhouses, orchards, warehouses, etc. commercialization structures) were almost demolished. Actually farms are extremely fragmentized, production units are too small, rural infrastructure is poor, which does not give the possibilities to have a big number of competitive agribusinesses oriented through commercial farming or exports (Rassas et al., 2012). So as consequence there is no uniformity in the quantity and quality of the products. Albania has considerable potentials for fruits and vegetables growth. Its climate-arable conditions are very adequate for successful growth for a wide range of fruits

and vegetables. Vegetable cultivation has increased by more than 40 % compared to 2000 (Statistical yearbook 2013). Fruit production had a greater decline through the first years of transition as a result of damages caused to the existing orchard. Production of fruits has almost tripled since 2000 (Leonetti et al., 2010). Still today, the pace of growth of this sector generally remains weak several reasons, which are already known and highly debated. Through years 1975 till 1990 Albania was an exporter of fresh vegetables. One third of the glasshouses production or approximately 50 000 tons with a value of 150 000 US Dollars was destined for export (Marku, 1999). The main vegetables for export were tomatoes and cucumbers. Actually the main area for vegetables production in Albania is the coastal and hilly area of central Albania, including Fier, Berat and part of Tirana region. Other important production areas are Shkodra (open field vegetables), Korca (potatoes and open field vegetables) and Lezha (melons). (Rural Development Strategy 2014-2020). Actually Albanian markets are dominated from domestic production, except in the winter months. The greenhouses production is not competitive with imported products during the winter season, for various reasons including high price of fuel for heating. At this point it is important to mention that imports have kept decreasing, and in 2012 and 2013 they were exceeded by exports (in quantity). The principal country for Albanian exports is Kosovo, which is followed from Monte Negro. For Albanian wholesalers is easier to contact directly with Kosovo intermediaries and the standards requirements are not so high. On the other hand in Monte Negro export farmers from Shkodra which is well-known for vegetables production. On the other hand the fruit processing industry is oriented towards jam production like cherry, peach, plum and figs. Meanwhile in preserving vegetables, pepper, cucumber beetroot, marinated cabbage, tomato for producing different sauces, carrots and also other vegetables, take an important place to produce pickles and also other processed food. The situation is optimistic because every year there is an apparent increase in the production of agro-industry and from year to year are being limited the imports, of these products compared to domestic production.

Materials and methods

Fruit and vegetables industry is considered as one of the most profitable in terms of capacity and at the same time one of the most problematic in terms of quality. The principal aim of the study is to give a detailed picture of fruit and vegetables sector in Albania and to identify the export growth opportunities. A survey was conducted with fruits & vegetables stakeholders. The instrument used for the survey was closed questionnaire. We made 23 face to face interviews with farmers, agro-food processing companies and wholesalers. The information was processed in a simple data base in Microsoft Excel. Through the database elaboration we will try to estimate the potentials for fruit and vegetables exports.

Results and discussion

As we mentioned above actually exist several problems that hamper exportation growth in Albania. The main duty of Albanian farmers and fruit and vegetables processors is the furnishing of the urban centres. The exportation is limited and is covered only from big producers of fresh fruit and vegetables. The absence of the economic structures in process of the collection and distribution of the products has encouraged the development of the

direct circuits of commercialization, from rural producers to intermediaries, or from agro enterprises to regional intermediaries.

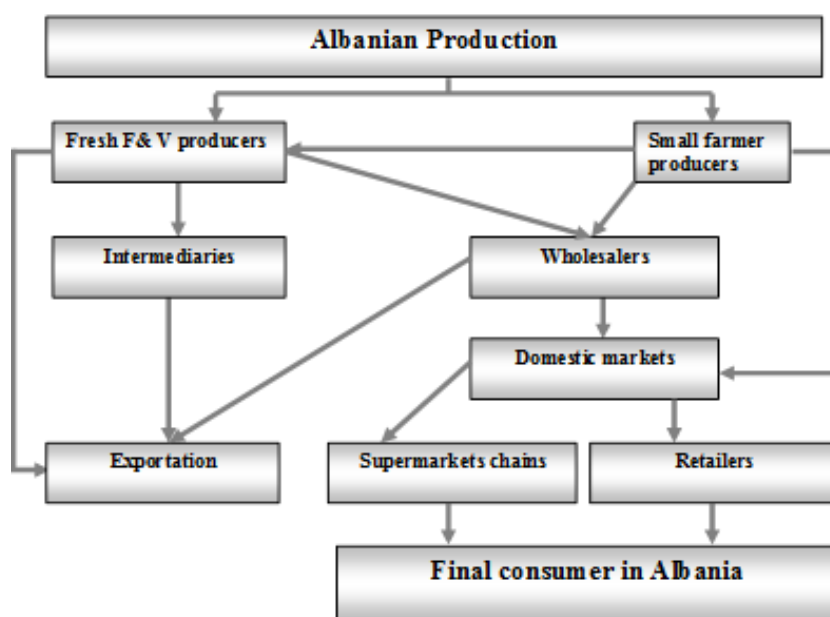


Figure 1. Circuits of Albanian production from the producers to final consumers.

Source: Estimation from questionnaires

So these kinds of structures are not sufficient for the circulation of the products and at the same time all the marketing channels are hurt with a lot of problems as techniques, economics, organizational etc. Albanian producers were not informed for the markets after the exportation of their products. Albania has a lot of opportunities to favorite exports, but at the same time some constraints.

The key elements, that can be mentioned who favor the Albanian exports are:

- Albania has a lot of autochton varieties and their promotion can be a very good export opportunity.
- Preserving a good image over agriculture products from the partners, who still look forward to import Albanian products (such as bio products).
- Favorable climate conditions (Vegetable production can be exported in countries such as Rusia, Lituania, Hungary, Poland etc).
- Low prices of agricultural products

Among the elements which are inhibitors of the Albanian agricultural exports, we can mention:

- The difficulty of organizing small producers, in order to adopt quality standards as required by target markets,
- Domestic market in Albania should unify the standards with export requirements (Small farmers sell not standartized products to local green markets in continuity).
- Albanian Government has no clear policies to support or subsidize export growth.
- Lack institutional mechanisms to facilitate standartization procedures. Near the Ministry of Agriculture is adopted the directory of standartization, which is the only institution that certificate agricultural productions.

- Problems linked with land property especially in Berati zone (Kutalli Village).
- Farmers are not market oriented, because they choose the seeds and fertilizants according to input distributors advices.
- Lack of specialized units and large scale to exploit the positive elements of the climate and the increase of earliness index of agriculture products.
- Post-harvest services (cold storage facilities, packing houses etc.) are limited.
- Limited cold storage facilities obstacles the assurance of a continuous demand for agricultural products. This is one of the main factors that increase the losses of products and at the same time price pass through a high volatility curve.
- In Albania the VAT (Value Added Tax) for inputs is high 20%, and this fact increase the price of fertilizers and also the price of agricultural products. Sometimes they prefer to use fertilizers imported from China, which are cheaper, but not standardized, therefore the quality of agricultural products is compromised.

On the other hand structural changes continue to transform the fresh fruit and vegetable exporting opportunity of Albania in regional markets and more. For this reason we decided to prepare a matrix which shows the presence of Albanian fresh products in regional, EU and other markets.

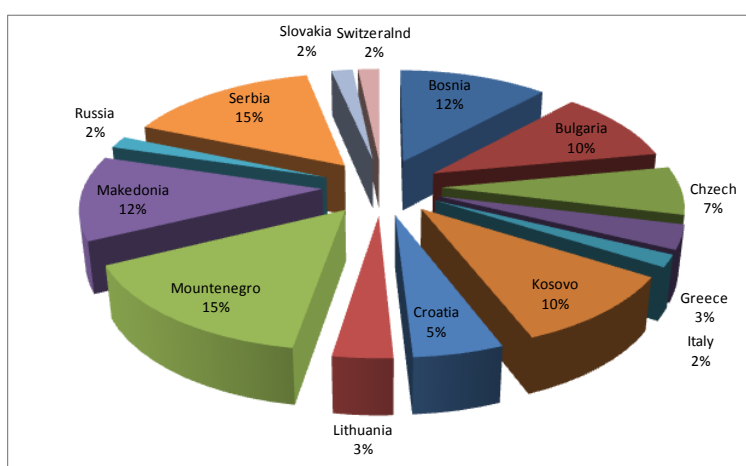


Figure 2. Export of fresh F&V according to interviewed stakeholders
Source: Our estimation

As we can observe from graphic the interviewed stakeholders export the main quantity of their fresh products in regional markets (Kosovo 10%, Monte Negro 15%, Serbia 15%, Macedonia 12%, B&H 12 %). Albanian products can penetrate easily in regional countries because we have acceptable standards requirements with them. The request for Albanian products arrives directly from regional wholesalers because they need to fill the gaps of their domestic products (problems due to seasonality, or to climacteric conditions). In the other side this fact shows the incompetence of Albanian producers to penetrate in EU countries, because of the lack of quality food standards and structured marketing channels. Usually the intermediaries from regional Countries especially from Kosovo and Macedonia prefer to avoid marketing channels and to gather the product directly from small farmers. This fact causes a lot of contradicts in local markets and at the same time with big producers of fresh F&V. This means that the principal market is Kosovo, because we export there in quantity and in volume. At the same time the Albanian producers gives

their expectancy for the export growth. According to their answers we tried to build an expectation matrix for Albanian products exports growth. This matrix shows the perspective of Albanian producers in near future, after their products have met the standards. We predict the values according to the actual size of their market share and according to the historical data that Albania had for the export of each product. The principal growth is sustained in two well known Albanian products: tomatoes and watermelon.

Table 1. Matrix of most prospects Albanian fresh products for exportation growth opportunities

Countries															Grand Total
Products	Bulgary	Greece	Hungary	Italy	Kosovo	Croatia	Lithuania	Mountenegro	Makedonia	Poland	Russia	Serbia	Slovakia	Switzerland	(%)
Tomatoes	8	2			1	3		1	0.5	3	2	2.5			23
Chestnuts				1									1	1	3
Cabbage	1						1								2
Cauliflower	1										1				2
Potatoes	1														1
Melon						2				1		1			4
Leek	0.5							0.5	0.5			0.5			2
Watermelon	8		5		3	8	2			17	4	7			54
Cucumbers	2				0.5			1	0.5		1	1.5			6.5
Grand Total (%)	21.5	2	5	1	4.5	13	3	2.5	1.5	21	8	12.5	1	1	11

Source: Our estimation

The total growth of Albanian exports according to our estimations is around 11%, because the expansion is limited from managerial competences to penetrate in foreign markets. The product standardization will increase the total costs of exportation and for this reason we can export products towards countries with less favorable climacteric conditions such as Poland, Ukraine etc. Those markets are very attractive but at the same time their requirements due to standards are higher. At this point it is important to mention that they are not familiar with the opportunities to use EU funds for upgrading standards and improve competitiveness. Even in case of familiarity with these Funds, they don't prefer to apply for them, because of their bureaucratic procedures and because of strong criteria requirements. The exportation growth for processed products is more difficult than for fresh ones. We can penetrate in short term perspective in niche markets only with traditional products such as conserved olives, olive oil and some types of marinades. The processors had a low economy of scale and for this reason they have very high transaction costs. Also for them another problem is the existence of a strong black market. They can't control the illegal production and the falsifications. This problem compromises their reputation not only in domestic markets, but also in international ones. So as conclusion we can say that Albanian market toward the processed products is very problematic and the exports expectations are very low.

Conclusions

1. Albanian producers and processors in lot of cases are incapable to penetrate in foreign markets, because in the great part of cases they don't have the right education and also it is difficult for them to act as international marketing managers. The first step to be taken is opening several intermediary offices (especially in Berat, Lushnje, Tirana, regions where the concentration of activities is higher) in Albania that should play several functions:
 - a. Link international markets with Albanian markets.

- b. To analyze international and national environment. (Market research analyzes).
 - c. Helping in distribution channels consolidation. Albanian products have high transaction costs and for this reason producers or processors usually prefer to send their products directly in supermarkets.
 - d. The survey indicated that there hardly existed any written contract between vendors and suppliers. Even the wholesalers do not use written contracts. They do not want long term commitment since trust and a ceiling market price do not exist. Liquidity risks will mitigated from the intermediation office for Albanian producers and suppliers.
 - e. Quality standards assurance for buyers. Actually farmers are advices from input distributors and for this reason exists a conflict of interest. They can't sell pesticides and at the same time advice the farmers. The office in collaboration with extension service, body certifications and universities can help farmers in this direction.
2. Albanian producers should assure continuity with regard to the quality and quantity of their production. In this case it will be very useful to encourage them to collaborate together only in terms of marketing. Previous experiences have shown that Albanian farmers can't operate together in terms of cooperatives and for this reason it will be an opportunity to help them to create marketing enterprises.
 3. Creation of a database in order to specify the Albanian producers and their products. If we have an updated map every year the processors will reduce the raw material costs and at the same time they will have complete information about the Albanian products and producers.
 4. Albanian producers and processors need to invest for label improvement or relabeling.
 5. Large scale companies are more likely to have full knowledge about EU grant programs. Meanwhile the small enterprises are not so much informed. But for both of them it is very difficult to apply in such structures because they don't have the necessary assets to do that. The difficulties are bigger speaking for Albanian producers. At the same time they have a lot of difficulties in business plans drawing and future predictions. The most important trainings we propose for them are:
 - a. Accounting for non accountants.
 - b. What will a lender want to know about my business
 - c. Necessary steps to draw a Business plan
 - d. Standardization systems and procedures.
 - e. Marketing strategies and planning

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BEHAVIOUR OF PLUM CULTIVAR 'KATINKA' UNDER CONDITIONS OF TROYAN REGION UNTIL ENTERING INTO FRUIT BEARINGGeorgiev Popski G.^{1*}¹Research Institute of Mountain Stockbreeding and Agriculture – Troyan

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Abstract

Since 2010, in the Research Institute of Mountain Stockbreeding and Agriculture - Troyan, the vegetative manifestation of the early ripening plum cultivar 'Katinka' has been researched until its entering into fruit bearing stage. The aim of the present study was to evaluate the behaviour of the cultivar under the conditions of Troyan region during the juvenile period. The vegetative and reproductive indicators of trees were studied. It was found that they had moderate growth and tight crown. The cultivar entered into fruit bearing early (in the second year after planting), as the first yield per tree was 0.5kg with average fruit weight of 25 g. Soluble solids content and total sugars in fruits were less than the standard cultivar 'Stanley'. The amount of tanning and anthocyanins was larger. Because of the high infection background of plum pox virus, which is characteristic for the region, only single symptoms were observed on leaves. The presence of virus was proved by DAS ELISA. The first results show that trees of plum cultivar 'Katinka' give fruit and develop well in the region of Troyan, and the cultivar is suitable for organic production of plum fruits.

Introduction

The region of Troyan is one of the main centers of plum production in Bulgaria and the results of the present cultivar study have a representative character.

The introduction of new plum cultivars in fruit production is favourable for time extension of fresh plum fruit presence on the market, and it also creates a variety for customers.

'Katinka' cultivar is a part of the selection of the institute in Hohenheim, obtained by crossing of Ortenauer x Ruth Gerstetter in 1982 by Dr. Hartman. The author defines it as tolerant to plum pox virus, as he bases himself on the hypersensitivity reaction that was inherited from 'Ortenauer' (Lichtenegger, et al. 2012).

Ondrášek, I. и кол., (2013), recommend it as being perspective for the conditions of Check Republic, along with 'Topking', 'Empress', 'Carpatin', 'Pitestean' and 'Valor'. Vávra (2012) studies the productivity of some German cultivars, including 'Katinka', which enter very early into fruit bearing and reach high yields under highly dense planting scheme. Due to the poor growth, similar results are announced by Blažek and Pištěková (2012).

Fruits become ripen early, in the end of July, or the beginning of August.

The aim of the study is to evaluate the behaviour of the cultivar under the conditions of Troyan region during juvenile period.

Material and methods

In 2010, in the Institute of Mountain Stockbreeding and Agriculture - Troyan, the early ripening plum cultivar 'Katinka' was studied, grafted on 'Fereley' rootstock, which was grown under 5x4 m scheme. Trees were planted in 2010 in 7 da plantation, on slopes and slightly productive gray forest soils, in trenches (ditches) – with stockpiled organic fertilization, without application of mineral fertilizers. They were formed as free growing crowns with minimum prunings. The following vegetative indicators were followed – trunk cross section (cm²), total growth (cm); and the reproductive indicators – Initial yield (kg), fruit and stone weight (g), and chemical composition of fruits.

Results and discussion

Climate and phenology

Figure 1 shows the tendency of average rainfall during vegetation for a ten-year period (2003/2013), and the actual rainfall amount during respective year. They have an influence over the growth rate of trees, flowering period, formation and ripening of fruits.

2011 and 2012 have similarities – drier summers, in 2013 the rainfall amount was greater in June, followed by drier months – July, August and September. In 2014, the rainfall amount during vegetative period was record high, especially for May, July and September.

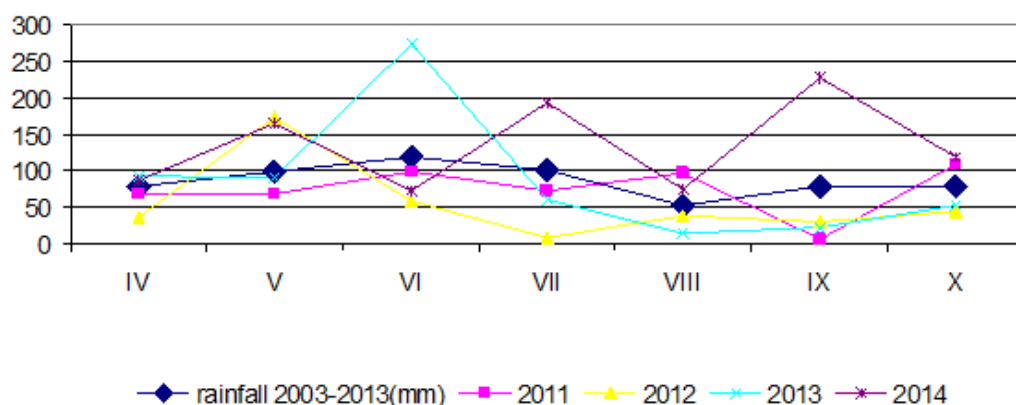


Figure 1. Rainfall amount for vegetative periods during the years of study

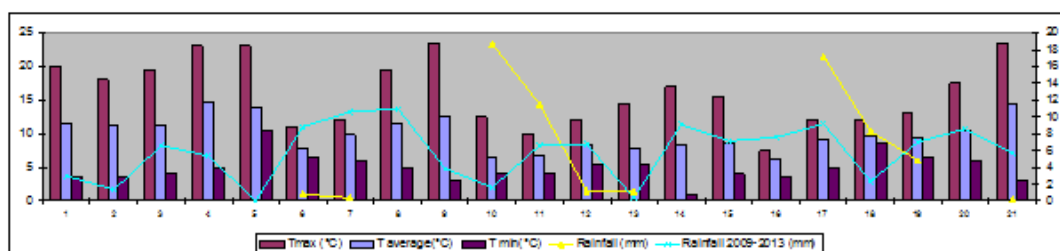


Fig. 2 Temperatures and rainfall values for April 2014

Flowering periods in 2014 for observed cultivars, along with 'Katinka', were rather different, but the end of the period for all of them was similar - 14.04. 2014. In our opinion, this is due to the reported high amplitude in the minimum and maximum twenty-

four-hour temperature, which caused the end of flowering period and compromised the whole 2014 harvest (Fig. 2).

According to a study of Milošević 2013, 'Hanita' and 'Jojo' are good pollinators for 'Katinka' cultivar. For the conditions in 2014, in the region of Troyan, these cultivars, along with the control cultivar 'Stanley', were in full blossoming simultaneously, but a little earlier than the flowering period of 'Katinka'. There is a coincidence in the flowering period among 'Katinka', 'C. lepotica' and 'Tor' (Table 1).

Table 1. Phenophases of flowering of plum cultivars from collection of RIMSA-Troyan in 2014

	Beginning of flowering	full flowering	End of flowering
Tegera	28.03-1.04	2-10.04	14.04
Stanley	30.03-2.04	3-8.04	9-14.04
Elena	30.03-2.04	3-8.04	9-13.04
Jojo	31.03-2.04	2-7.04	8-14.04
Hanita	1-4.04	3-8.04	9-13.04
C. lepotica	2-5.04	6-12.04	12-14.04
Top	4-8.04	8-13.04	14.04
Katinka	6-8.04	8-12.04	14.04

Growth

Vegetative indicators, followed during the experimental period, showed that trees of 'Katinka' cultivar, when grafted on rootstock of 'Fereley' cultivar, manifested moderate growth. The increase of trunk was about 10 cm² per year, similar to 'Stanley' at the same age. Total growth (measured on a marked branch), was compared in years because of the influence of the climate factors. During the study period, 'Katinka' cultivar showed almost equal growth in relation to the total growth, as it was the greatest in 2014 (652 cm), when there was no fruit harvest and there was abundant rainfall amount, and in the dry year 2012, the annual growth was 350 cm (Table 2). For 'Stanley' this indicator varies more widely, as in 2013 it was the lowest, and in 2014 (586 cm), a little less than 'Katinka'. 'Katinka' cultivar could be recommended for higher density planting and intensification scheme, because of its tight crown, which is formed similarly to 'Čačanska lepotica'.

Table 2. Vegetative manifestations - Total growth (cm)

	2011	2012	2013	2014
Stanley	670.0	556.0	85.0	586.6
Katinka	496.3	349.6	503.3	652.0
LSD 0.05				9.75

Fruit bearing

The first fruits were obtained on the second year after planting. They had fresh fruit flesh, and dark blue fruit skin. The fruit weight was about 20 to 28 g, and the stone was about 4-6% of it (Table 3).

Table 3. Reproductive manifestations of cultivar 'Katinka'

	Fruit weight (g)	Fruit stone (g)	Fruit stone (% of the fruit)	Yield (kg/tree)
2011	20,28	1,28	6.31	0,50
2012	22,54	1,30	5.76	0,54
2013	24,77	0,94	3.79	1,35

The chemical composition of fruit is entirely influenced by the climate conditions of the respective year. In 2012, which was dry, the accumulated dry matter and total sugars were the most for both cultivars and respectively the acids were least. As a varietal characteristic for 'Katinka', however, could be seen that it accumulates significantly less dry matter and less sugars in comparison with 'Stanley' (probably because of its earlier ripening period) (Table 4).

Table 4. Chemical analyses of fruit

	Soluble solids (%)	Total sugar (%)	Inverted sugar (%)	Sucrose (%)	Total acids (%)	Tannins (%)	Anthocyanins (mg/%)
2011							
Katinka	16,50	8,93	4,85	3,88	0,60	0,144	28,39
Stanley	18,75	9,75	7,70	1,95	0,34	0,040	4,52
2012							
Katinka	17,00	9,20	8,20	0,95	0,64	0,083	5,48
Stanley	27,90	12,80	8,55	4,04	0,53	0,309	18,23
2013							
Katinka	11,75	4,85	4,85	0	0,74	0,085	6,29
Stanley	14,50	11,30	7,20	3,90	0,87	0,042	5,48

Behaviour to plum pox virus

Under the conditions of the region of Troyan, at high infection background of plum pox virus, 'Katinka' cultivar showed symptoms on leaves and proved the presence of the infection by ELISA test. There are no symptoms on leaves and it has not been found out whether there is economic significance for the quality of fruits and the yield.

Malinowski et al. 2013, define 'Katinka' as sensitive to the conditions of Poland. It is found out that for the region of Poland, this cultivar shows sensitivity to the plum pox virus (Milusheva, 2014).

Conclusions

1. Results from the study of 'Katinka' cultivar, for the period until entering in full fruit bearing, show that this cultivar gives fruit and develops well in the region of Troyan, and it is also suitable for organic production of plum fruits.
2. The moderate growth rate, manifested by 'Katinka', gives the opportunity to apply semi-intensive planting schemes, which are economically more effective.
3. 'Katinka' cultivar shows symptoms of plum pox virus on leaves even in the second or third year after planting, but there are still no symptoms on fruits.

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**EFFECTS OF PRD, AND MULCHING ON FRUIT QUALITY
PARAMETERS OF "POLKA" RASPBERRIES**Lepaja K.¹, Kullaj E.¹, Lepaja L.¹, Avdiu V.²¹Agriculture University of Tirana, Koder-Kamez, Tirana, Albania,²University of Prishtina, Faculty of Agriculture and Veterinary, Kosovo

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Abstract

The aim of this research was to determine the productive response of 'Polka' raspberry canes after the application of partial rootzone drying (PRD) combined with mulching from end of the August to end the September. Using a water budgeted methodology, four levels of irrigation, specifically 100% of ET (control) standard lateral, with two laterals (TL), side lateral (SL) and without irrigation (WI), were applied to 10 canes, 5 of which were mulched (wood chips) by a 10 cm layer. The experiment was conducted in Kosovo (Podujeva region) during 2014 on a raspberry orchard of 1 ha on first year planting using a nested experimental design. Using ANOVA two-way with post hoc testing we found that irrigation had significant changes in fruit diameter, fruit length, fruit weight and total yield. PRD and mulching had a combined effect on fruit weight, while changes were not significant for number of fruit. Mulching had positive effect on all parameter values. Considering the young age of orchard, first year of application of PRD and long - term consequences of water stress experiment is continuing.

Key words: *Rubus idaeus*, 'Polka', water stress, drip irrigation, mulch.

Introduction

Through their root system plants receive nutrients dissolved in water thus reduction of water absorbed by the roots reduces mineral uptake. Drip irrigation, used also in our experiment is the most effective irrigation system and its application in fruit tree production is spreading around the world after its first discovery in Israel. Drip irrigation enables rational use of water, to wet the surface only in the vicinity of the tree (but allowing the humidity to expand in-depth), reducing the soil evaporation. The uniform distribution of water for each tree cannot be achieved with other types of irrigation. In addition, other advantages of this way of irrigation are: application in different terrains, soil wetting, prevention of crust formation, free access for people and machinery after every irrigation event, avoidance of soil compression, prevention of erosion, possibility for the use of fertigation, etc (Lepaja *et al.*, 2015). Production of raspberry considered (*Rubus idaeus*) that is of particular importance for the economy of Kosovo. Until now about 500 ha are planted with raspberry. Water shortages in the territory of Kosovo, especially during the vegetation period, necessitate intervention with supplemental irrigation, but the application of PRD or RDI can start from the beginning or middle of June due to the fact that Kosovo has sufficient rainfall during May. Raspberries have shallow root system and

aren't endurable to moist and dry soils, therefore care should be taken regarding irrigation by making an irrigation program depending on climate conditions namely precipitation and soil moisture measurements with relevant instruments (irrometer). Drip irrigation system must be used for watering raspberries. Controlled alternate partial root-zone irrigation (CAPRI), also called partial root-zone drying (PRD) in other literature, is a new irrigation technique and may improve the water use efficiency of crop production without significant yield reduction (Kang and Zhang, 2004), then PRD is a method for minimizing water use with little or no negative effects on fruit growth but causes a growth inhibition useful shoots (Kullaj, 2008) cit. Lepaja *et al.*, 2015. The quantity of water in raspberry production is important throughout the life of the planting. Too much water over a long period of time on poorly drained soil will cause plant root disease and plant loss. Climatic conditions determine water needs. When calculating the amount of water needed for raspberries using drip irrigation, only the root zone area needs to be irrigated. Drip irrigation applies water to a part of the root zone and does not broadcast the water as an overhead or flood method does, so it allows more efficient application of water to the desired crop (Funt and Ross, 2013). There was clear evidence from the field experiment that the baseline irrigation supplied by the farmer was suboptimal with additional irrigation enhancing growth, yield and fruit size. There was some evidence that the PRD treatment even enhanced yield while it simultaneously reduced vegetative growth (Stoll *et al.*, 2002; Dry *et al.*, 1996, 2000; Spreer *et al.*, 2006). Pot experiments with split-root plants and two years of field experiments with manipulation of soil water content have shown that partial rootzone drying (PRD) does not affect yield in raspberries, *Rubus idaeus* L. 'Glen Ample' and 'Glen Prosen'. In some cases PRD can reduce stomatal conductance, thus reducing water loss (Grant *et al.*, 2004). According to Downer (2009) there are several reasons to apply mulch. Mulches prevent weeds from germinating, reduce evaporative loss from soil surfaces, add organic matter to soils thereby increasing their mineral content, increase soil-borne disease suppression, and finally as shown in some studies, increase the growth of trees (Downer, 2009; Greenly and Rakow, 1995; Lučić *et al.*, 1996). The objective of this study was to determine the impact of PRD in combination with mulching on quality parameters where water resources are limited, and canes are in water stress.

Materials and methods

This field experiment was designed to assess the productive response (fruit quality parameters) of 'Polka' raspberry canes after the application of partial root zone drying (PRD) combined with mulching, were used in a commercial raspberry orchard. The experimental set up was a nested design, with canes of cv. 'Polka' raspberry. Four levels of irrigation were applied, 100% of evapo-transpiration (ET) as control standard lateral (one lateral), with two laterals, side lateral and without irrigation. Orchard is planted on April 2014. Drip distance in the lateral pipe was 0.20 m while drip irrigation spend 1.6 litres of water/h per drip. A total of 20 irrigations (one irrigation is made per 1 hour 30 min) were applied. Partial root zone drying (PRD) is started to applied from July until end of the September, but our parameters was measured from 24.08.2014 until 21.09.2014. Each treatment (each level of irrigation) has been in a row. For each treatment we used 10 canes, 5 of which were mulched at with a 10 cm thick layer of wood chips, totalling 40 canes for the entire experiment. Mulching material was placed in a row of a width of 0.80 m on July 20, 2014. Planting distances were 3 m between the rows and 0.40 m in the row. All fruits

in all canes were counted. Their size (diameter and length of fruits) was measured (in mm) at the equator with a calibre using all fruits per canes, all the time during every harvest. Average fruit weight was measured (in grams) using an analytical balance for all fruits, each harvest. Yield (in g/cane) was calculated at all period of the harvest time measuring the total weight of all fruits per cane. Our state has a moderate continental climate with a coastal impact which penetrates through the valley of the Drini i bardhë moderating markedly continental climate elements (Lepaja *et al.*, 2014; 2015). In Kosovo average temperature multiyear (1951-1980) is 10.3 °C, that of vegetation 16.5 °C, the coldest month is January (-0.9 °C) while the hottest month is July with 20.1 °C. Regarding the annual rainfall is 744.8 mm, and during vegetation is 346.7 mm which shows the need to intervene with supplementary irrigation (Zajmi, 1996). Water shortages in the territory of Kosovo, especially during the vegetation period, need supplemental irrigation.

The amount of rainfalls for Prishtina region (Podujeva) for a 30-year period are 608 mm and 325.9 mm during the growing season. Rainfalls during the period of the study were much lower compared to the average 30-year period with a total of 892.7 mm and 624.4 mm during the growing period. The first irrigation was applied at the end of May when temperatures started to raise and there were no rainfalls. The temperature during the vegetation period were 17.01 °C. The average temperature and the temperature during the growing period was 1°C higher compared to the 30-year average. Data from the measurements were analysed using ANOVA two-way with post hoc testing.

Results and discussion

We found significant changes in a series of reproductive parameters which confirms the results of other authors (Davies *et al.*, 2000). All value of productive parameters are more higher in two laterals treatment. The results of the research (ANOVA) showed that irrigation had significant changes in fruit diameter, fruit length, fruit weight and total yield. PRD and mulching had a combined effect on fruit weight, while changes were not significant for number of fruit. Our results of the application of RDI and mulching on the quality parameters of Polka raspberry with differences between treatments according LSD testing are presented in table 1.

Table 1. Average data for reproductive parameters with differences between treatment according LSD testing

Parameters		No of fruits	Diameter of fruit (mm)	Length of fruit (mm)	Fruit weight g.	Total yield g./cane
Normal irrigation	Mulch +	14.85	a 21.17	a 25.36	a 4.55	a 67.32
	Mulch -	15.23	21.50 a	25.74 a	4.85 a	74.05 a
Two laterals	Mulch +	17.12	a 21.60	a 25.49	a 4.66	a 79.67
	Mulch -	16.63	20.40 b	25.09 b	4.55 b	74.66 a
Side laterals	Mulch +	14.28	b 20.44	a 24.86	b 4.20	b 59.30
	Mulch -	14.33	20.28 b	24.41 c	4.10 c	59.08 b
Without irrigation	Mulch +	14.03	c 19.37	b 23.47	c 3.55	b 49.20
	Mulch -	13.92	19.07 c	22.60 d	3.20 d	45.21 c

* Letters on the left in each column represent differences for mulch +, while on the right represent differences for mulch- (without mulch).

Concerning some parameters researched by the authors (Stoll *et al.*, 2002) our results are consistent with the authors concerned. On two laterals best results are achieved under the use of mulch. After two lateral treatment, approximate values are reached to the variants normal irrigation and side laterals, and lastly the treatment without irrigation.

Effect of partial rootzone drying reduction a vegetative growth without being influenced in the productive parameters (Stoll *et al.*, 2002; Dry *et al.*, 1996, 2000; Spreer *et al.*, 2006; Grant *et al.*, 2004).

Table. 2. Total yield g./cane, ANOVA

Irrigation (A)		Mulching (B)			Average (A)
		Mulch +	Mulch -		
Normal irrigation		67.32 a	74.05 a		a 70.68
Two laterals		79.67 a	74.66 a		a 77.16
Side laterals		59.30 b	59.08 b		b 59.19
Without irrigation		49.20 b	45.21 c		c 47.20
Average (B)		63.87ns	63.25 ns		Interaction A x B ns
Factor		A	B	A x B	B x A
LSD	1%	15.7259	11.4773	22.3647	22.9546
	5%	10.9452	8.1863	15.8336	16.3725

The higher difference of total yield g./cane as it is shown in the table 2, where two laterals is 79.67 g./cane in mulch treatment, while without irrigation mulch – is 45.21 g./cane.

Value in two laterals (mulch + and mulch-) are more higher than another treatment, but in treatment without irrigation total yield was under 50 g./cane. In the table it is shown also differences between treatment (ANOVA). To treatments with mulch in average there were no statistical differences, but compared within the same treatment differences existed where to mulch + treatment without irrigation there were differences in the level of 0.01% with normal irrigation treatment and two laterals, while to treatment mulch - side laterals and without irrigation had differences with the two other treatments at the level 0.01%, but also side laterals and without irrigation between them had the same difference at the level 0.01 %.

Mulching had positive effect on all parameter values (Lepaja *et al.*, 2014, 2015; Downer, 2009; Greenly and Rakow, 1995; Lučić *et al.*, 1996).

Conclusions

Water is the limiting factor in the production of raspberries. Raspberries is sensitive to drought but is sensitive to excess water also, therefore should be irrigated frequently but with smaller quantities of water. Placement of instruments for measuring the moisture in the soil will greatly facilitate management of irrigation so the water would be spared, but will also increase production. Our country during vegetation has lack of rainfall, the application of the PRD, is an advantage. Application of partial rootzone drying respectively four different levels of irrigation (normal irrigation, two laterals, side lateral and without irrigation) in raspberry in combination with mulch is a new thinks (innovation), and each result will contribute to the knowledge of this culture and PRD.

At the first our results in first year of orchard and application of PRD can be obtained primarily as a result of weather conditions: temperature and rainfall during the

time the experiment, furthermore long-term effects of deficit irrigation, together with climatic conditions, crop techniques variations, type of soil, age of plants etc. must be considered, because the long-term plant responses to PRD or RDI are more accurate than short-term responses (Lepaja *et al.*, 2015).

Based on our investigations on the optimal deficit irrigation regime under the agroecological conditions of Kosovo, and Podujeva in particular, under an intensive raspberry growing technology we demonstrated that irrigation, mulching and their combination have affected the measured parameters.

At the end of the treatment period, of PRD application combination with mulch, we found changes in a series of productive parameters (fruit quality parameters). We found that irrigation had significant changes in fruit diameter, fruit length, fruit weight and total yield. PRD and mulching had a combined effect on fruit weight, while changes were not significant for number of fruit. Mulching had positive effect on all parameter values. At the first year of experiment the use of mulch at 10 cm thick layer has influenced most of the productive parameters and there was no weed. All value of productive parameters are more higher in two laterals treatment.

Considering the young age of orchard, first year of application of PRD and long - term consequences of water stress experiment is continuing.

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UDC: 634.14-152.64(497.2)
Original scientific paper

STUDY ON SOME ECONOMIC QUALITIES IN DIFFERENT GENOTYPES OF *CHAENOMELES* SP.

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Abstract

The study was conducted during the period 2008-2012 in the collection plantation of the Research Institute of Mountain Stockbreeding and Agriculture-Troyan (RIMSA). Six genotypes of *Chaenomeles* sp., distinguished by a series of valuable economic qualities, were object of study. The following indicators were determined: phenological calendar, fruit weight (g) and yield per shrub (kg). The genotypes S5 and S3 were the first to enter into vegetation. They were also distinguished by the longest flowering phase. The largest genotypes in size were S4 - 60.23 g and S6 - 59.70 g, and the smallest fruits had genotypes S1 - 24.59 g and S2- 34.79 g. The highest average yield per shrub was obtained in genotype S4 - 5.59 kg and genotype S3 - 5.08 kg.

Key words: *Chaenomeles* sp. phenology, fruits, yield

Introduction

In the conditions of changing climate, Japanese quince is a culture that deserves attention because of its increased resistance to cold and drought.

In relation to the environmental pollution, increase of morbidity in the population, plants rich in biologically active substances having adaptogenic, antimutagenic, immunomodulating, geroprotective character, are particularly topical. The species and hybrids of *Chaenomeles* sp. Lindl native to China, Japan and Tibet are one of the perspective sources of such substances. *Ch. japonica* and *Ch. x superba* (Frahm) Rehd are the most widespread of them due to the high degree of resistance to cold and drought. They are characterized by medium-sized fruits reaching in separate genotypes weight 50-100g (Меженский, 1989; Abbott, 1999; Nedviga *et al.* 2003).

The fruits of Japanese quince occur to be valuable raw material for the food processing, pharmaceutical and perfume industry, due to its rich chemical composition (Komar-Temnaya *et al.*, 2001; Mezheniskij, 1996).

Japanese quince can quickly and cheaply provide raw material for industry, as it meets all the requirements of the contemporary ecological production. *Chaenomeles* sp. Lindl is characterized by early onset of fruit-bearing (even in the 2nd year), comparatively high yield (up to 2-10 kg per shrub), high ecological plasticity, resistance to pests and diseases, low cost of production (Nedviga, 1994; Мондешка, 2005).

Materials and methods

The study of the available genetic resources in RIMSA-Troyan was conducted during the period 2008-2012 in the collection plantation of *Chaenomeles* sp. six genotypes from seedlings were subject of the present study. They were obtained by open pollination, having valuable ornamental, medicinal and nutritional qualities.

Soil preparation of areas during the creation of new plantations was carried out by trench method (local stocking organic fertilization). Planting scheme was 2,5m/1m. The shrubs were grown at conditions without irrigation, natural grassed inter rows, mowed several times depending on the rainfall during the vegetation period. The data analyses are made by Лидански (1998).

The following indicators were studied:

Phenological calendar:

- onset of vegetation;
- onset of flowering;
- full flowering;
- end of flowering;
- picking ripeness of fruit;
- harvest.

Reproductive indicators:

- average fruit weight (g);
- yield per shrub (kg).

Results and discussion

The selected genotypes of *Chaenomeles* sp. entered into vegetation at same time. The earliest bud bursting on 14th January was registered in genotype S5 in 2008 and on 28th February –in 2010, and the latest – on 1st April in genotype S1 in 2012. The early warm weather in the first months in 2008 (the average month temperature in March is 7,5 °C) is reason the observed six genotypes to enter into vegetation at the same time – from 5th to 10th March (Table 1).

In 2009 and 2010 this indicator varied within wider limits. The onset of vegetation was the earliest in genotype S5 – from 28th February (2010) and 5th March (2009), to 20th March in genotype S2 (2009). In the last two years of the experiment five of the genotypes (except S5) enter into vegetation at the same time at the end of March and on 1st April (Table 1).

Genotype S5 approved to be early flowering because in all five years of the experiment it first began to bloom – till 11th April. It was followed by genotype S3, which flowering, except in 2008, began in the second ten days in April (Figure 1).

Table 1. Phenology of selected genotypes of *Chaenomeles* sp.

Genotypes	Year	Onset of vegetation	Onset of flowering	Full flowering	End of flowering	Picking ripeness
S1	2008	10 th March	15 th April	26 th April	11 th May	15 th October
	2009	18 th March	22 nd April	25 th April	15 th May	7 th October
	2010	13 th March	26 th April	29 th April	14 th May	7 th October
	2011	28 th March	19 th April	27 th April	10 th May	3 rd October
	2012	1 st April	21 st April	27 th April	3 rd May	24 th September
S2	2008	10 th March	16 th April	24 th April	12 th May	15 th October
	2009	20 th March	22 nd April	25 th April	12 th May	7 th October
	2010	12 th March	23 rd April	28 th April	15 th May	7 th October
	2011	28 th March	19 th April	27 th April	10 th May	3 rd October
	2012	1 st April	26 th April	29 th April	5 th May	24 th September
S3	2008	5 th March	7 th April	20 th April	10 th May	5 th October
	2009	8 th March	14 th April	20 th April	16 th May	7 th October
	2010	8 th March	10 th April	19 th April	17 th May	7 th October
	2011	3 rd March	12 th April	18 th April	5 th May	1 st October
	2012	27 th March	19 th April	27 th April	10 th May	24 th September
S4	2008	9 th March	15 th April	22 nd April	10 th May	17 th October
	2009	18 th March	20 th April	24 th April	11 th May	26 th October
	2010	9 th March	26 th April	29 th April	13 th May	26 th October
	2011	26 th March	14 th April	25 th April	10 th May	29 th October
	2012	27 th March	18 th April	25 th April	3 rd May	1 st October
S5	2008	14 th Января.	6 th April	16 th April	10 th May	15 th October
	2009	5 th March	6 th April	22 nd April	11 th May	20 th October
	2010	28 th February	6 th April	18 th April	11 th May	20 th October
	2011	15 th March	10 th April	19 th April	6 th May	29 th September
	2012	20 th March	11 th April	24 th April	3 rd May	1 st October
S6	2008	10 th March	13 th April	24 th April	10 th May	29 th October
	2009	13 th March	20 th April	23 th April	12 th May	10 th October
	2010	11 th March	13 th April	19 th April	13 th May	10 th October
	2011	27 th March	22 nd April	28 th April	10 th May	3 rd October
	2012	29 th March	16 th April	25 th April	2 nd May	24 th September

In the rest genotypes the onset of flowering started earlier only in 2008 because of the already mentioned earlier warming of weather in March. In April the same year the average day temperature was very high – 11,4 °C, which defined it as one of the two years (and 2012 – 12,2 °C) of the experiment with the warmest winter-spring months.

Flowering started in the second half of April in all years of the experiment in genotypes S1, S2, S4 and S6 with few exceptions.

The period of full flowering from the initial began three days after the opening of the first blooms in genotype S1 (in 2010); S2 (in 2009 and 2012); S4 (in 2010) and S6 (in 2010) to thirty days in genotype S3 in 2008 and S5 in 2012. Only in genotype S5 in 2009 the full flowering started 16 days after the onset of flowering.

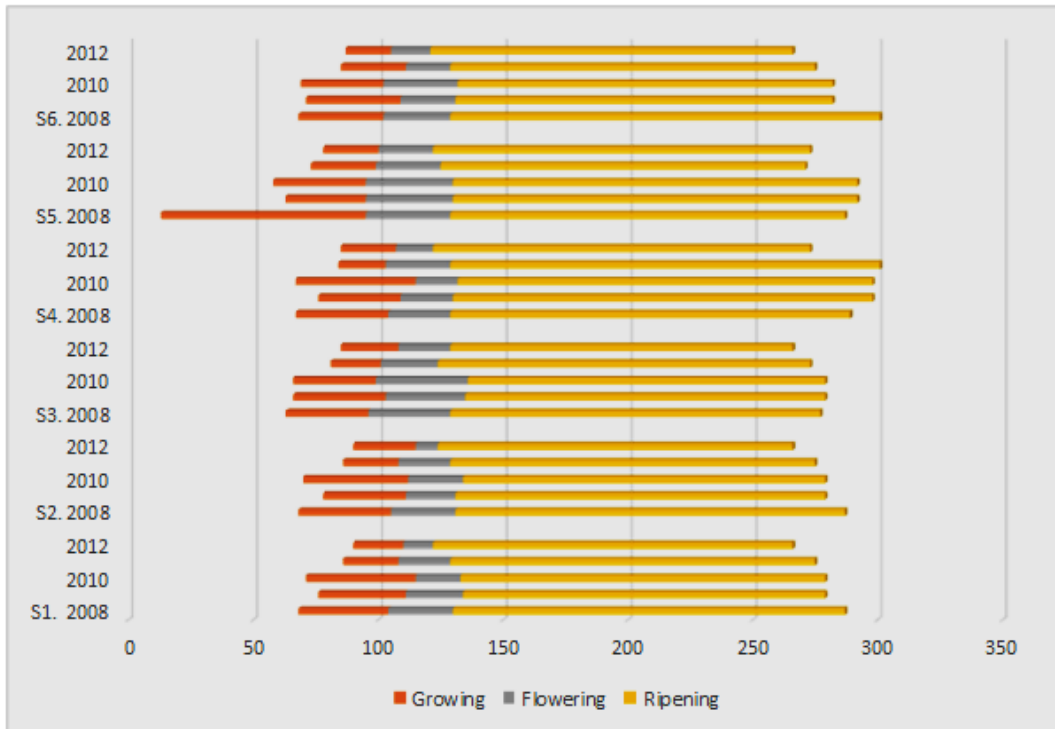


Figure 1. Phenogram of selected genotypes of *Chaenomeles* sp.

Fruits from genotypes S1, S2 and S3 reached picking ripeness in the first ten days of October in 2008, 2009, 2010 and 2011. They ripened at the end of September only in 2012 because in July and August that year the average month temperature reached the record-breaking values of 24,2 °C and 32,3°C, respectively. In the rest three genotypes – S4, S5 and S6 fruits were harvested 3-4 weeks later. For example, genotypes S4 and S5 were picked on 26th and 29th October in 2009 and 2010, respectively, and genotype S6 on 29th October in 2008. It should be noted that some conventionality exists in the determination of picking ripeness of fruits as it is accepted that the seed coat turns dark brown, the skin turns yellow 2/3 of the fruits and they become fragrant. After that takes place a period of post-harvest ripening in which all fruits acquire typical color and flavor of genotype. Fruits of Japanese quince in the investigated genotypes are distinguished by shape, colour and size. With regard to fruit weight genotypes S4 and S6 were characterized with the highest average weight of fruits (about 60g). The smallest in size were genotypes S1 and S2 (24,59g and 34,79g, respectively) (Figure. 2).

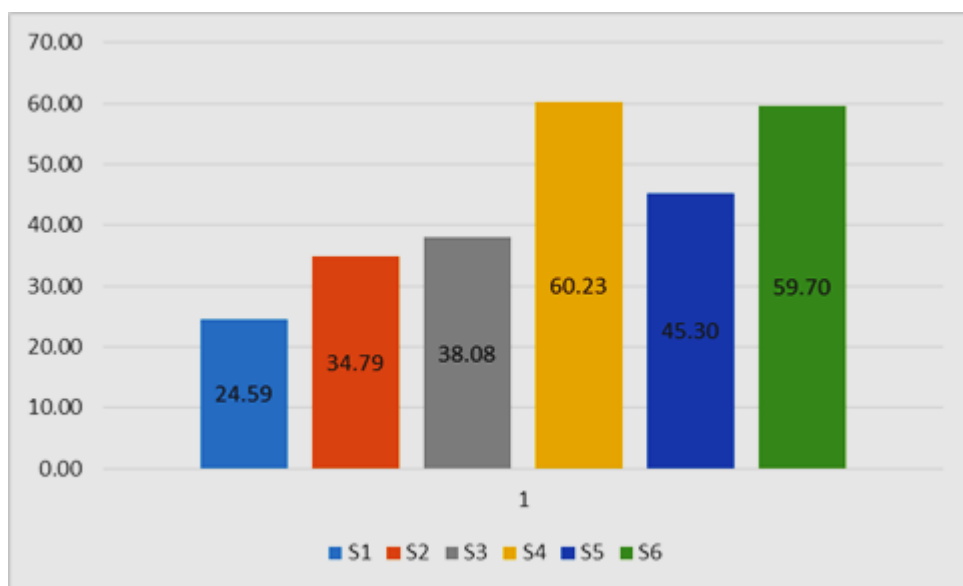


Figure 2. Weight of fruits of the studied genotypes of *Chaenomeles sp.* (g)

The average yield of fruits per shrub for the studied period is shown in Figure 3. Record-breaking yield was obtained in genotype S4 in 2009 – 22,2 kg. Probable reason for this is the month precipitation in September 105,5 l/m², which is far above the average for this month, observed for the previous 40 years period. The highest average yield for all the studied period was registered in the same genotype S4 – 15,590 kg., and the lowest 10 times less – in genotypes S1 and S2 – to 1,5 kg. Genotype S3 was also highly fruitful– with 5 kg., followed by S5 – respectively with 4, 64 kg and medium fruitful genotype S6 – 3,37 kg. The genetic specifics are very well proven mathematically (LSD_{0.001}–4.58, fig. 3).

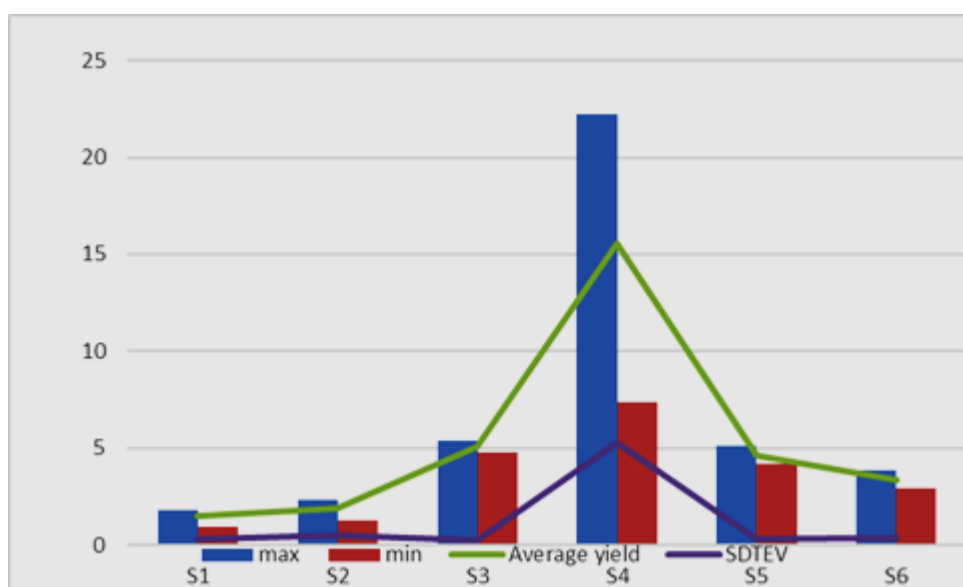


Figure 3. Yield from a shrub of the studied genotypes of *Chaenomeles sp.* (kg)

* Analysis of variance (ANOVA): LSD_{0.05}–2.57 LSD_{0.01}–3.46 LSD_{0.001}–4.58

The variation in yield over the years in some genotypes was in very low values (S5 and S6), and much higher in S2 (25,07%) and S4 (33,85%). Alternativeness is not observed in fruit-bearing and lower values of the indicator are associated with late summer drought immediately before harvesting the fruits.

Conclusions

Genotypes of *Chaenomeles* sp. Lindl considerably differ in the studied indicators. The reproductive parameters of fruit weight and yield per shrub varied within wide limits. The interval between genotypes is long in the occurrence of phases: onset of vegetation, flowering and harvest of fruits.

Genotype S4 is distinguished as highly fruitful, and genotypes S3, S5 and S6 are moderately fruitful.

The most perspective genotype from all the studied genotypes for the conditions of Troyan region is distinguished genotype S4.

The results of the investigation give us reason to conclude that culture *Chaenomeles* sp. grows and fruitbears normally in Central Balkans Mountains and is relatively undemanding to soil and plant protection.

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MICROPROPAGATION OF LINGONBERRIES (*VACCINIUM VITIS-IDAEA* L.)

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Abstract

The interest towards lingonberries has increased during the last years, because their high levels of phenol acids and flavonoids in their fruits, which have a relation toward the physiological processes in the human organism. One of the limiting factors for their growing is the lack of planting material. The classical methods for planting material production through cuttings are not effective for them, because of their low rooting ability. Biotechnological methods guarantee the obtainment of high qualitative, homogenous planting material without viruses. The main disadvantages of *in vitro* technology are the high price of planting material, the need of highly professional personnel and expensive equipment. Axillary buds from intensively growing plants of lingonberries were cultivated on modified WPM, which consisted of 4 mg/l zeatin and 5 mg/l 2-iP. The influence of plant growing regulators was evaluated after 4 weeks. The highest multiplicative potential was obtained at the second passage – 4.46 items, and the highest was the length of micro shoots of the fifth passage from the research – 3.38 cm. Successful rooting (75 %) of micropropagated plants of *Vaccinium vitis idaea* L. was accomplished at WPM, enriched by 1 mg/l IAA

Key words: lingonberry, *Vaccinium vitis idaea* L., micropropagation, zeatin, 2-iP

Introduction

The scientific research interest in growing small-sized fruit species increases worldwide. This is due to their wide spread, early ripening, plasticity and their extremely valuable nutritional and medicinal qualities. Organic cranberries are suitable both for fresh consumption and processing in various healthy foods, which help for decreasing cholesterol, for disinfection of kidneys and bladder. They are also used for stomach disorders due to their high content of arbutin (Novelli S., 2003). The aim of present study is to develop a system for accelerated propagation of lingonberries (*Vaccinium vitis-idaea* L.) cv. Miss cherry and obtaining of homogeneous pre-base planting material.

Materials and methods

Introduction in in vitro culture

This working protocol was developed in the Laboratory for micropropagation of fruit tree species in RIMSA, the town of Troyan. For source explants were isolated axillary buds of lingonberries (*Vaccinium vitis idaea* L.), cv. Miss cherry which had been pre-washed in

running tap water, with addition of 1-2 drops of Tween 20. The sterilization system included treatment with 70° spirt for 30 seconds and 0.1 HgCl₂ for 3 min, followed by triple washing by distilled water.

Multiplication

Multiplication stage was carried at WPM (McCOWN et al., 1981) basic nutritional medium, MS vitamins with a supplement of 4 mg/l zeatin and 5 mg/l 2-ip. The specified nutritional medium gave a good result in micropropagation of high bush blueberry (Georgieva et al., 2008). Sucrose - 20 g/l was used as a carbohydrate source. The acidity of the medium was corrected to 4.2 prior to autoclaving of 1.2 atm and 121° C for 10 min. Proliferation stage continued up to 6 passage as the duration of subcultivation was 28 days.

Rooting

For the successful rooting of lingonberries was tested 1/2 WPM nutritional medium, consisting of 1 mg/l IAA. All *in vitro* plants were cultivated in phytostatic chamber at a temperature of 22±2 °C, illumination 2500 lx and photoperiod 16/8 hours. Rooting experiments were conducted in spring (February/March).

Adaptation

Adaptation of *in vitro* plants with a height of stem to 3 cm and well-developed root system was carried in spring - April and May, in the course of 40 days. The substrate, used for adaptation, contained acid peat. Acclimatization was done under polyethylene at high air humidity (75 %) and a temperature of 20 - 22° C. The average number and length of shoots in different passages of cultivation were assessed. Rooting percentage, average number and length of roots were reported during the stage of rhizogenesis.

Results and discussion

The comparative analysis of results showed that the interaction between growth regulators, the basic nutritional medium and passage of cultivation were favourable for growth induction for lingonberries (*Vaccinium vitis-idaea* L.) cv. Miss cherry. In Figure 1 is depicted the generative potential in separate passages of cultivation of the tested cultivar. The results showed very strong shooting proliferation of the second passage - 4.46 items from explant. There were a reduction of multiplication potential from third to sixth passage of cultivation, where it was 1.85 items. The observed genotype dependence was probably due to the interaction among basic nutritional medium, growth regulators and genotype of lingonberries.

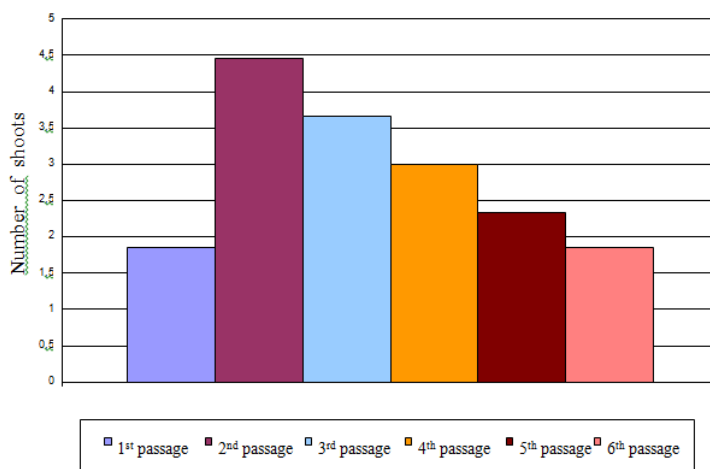


Figure 1. Multiplicational potential of lingonberries (*Vaccinium vitis-idaea* L.) cv. Miss cherry

The analysis of results showed that the average length of shoots varied from 2.04 (second passage) to 3.38 cm (fifth passage) (Figure 2). There was a certain relationship among the length of obtained shoots and their number - for example, the passages, whose micropropagation potential was lower, their shoots had the greatest length. In their experimental work in micropropagation of lingonberries, Debnath, (2001) obtained very good proliferation in using 2-ip and zeatin, as they found that zeatin was more effective cytokinin and it induced 2-3 times more shoots in "Regal" cultivar. This experimental protocol proves that $\frac{1}{2}$ WPM with supplement of 1 mg/l IAA induced 75% rhizogenesis in lingonberries. 1.45 average length of roots and 4.52 average number of roots for the studied cultivars were reported. Similar to our results in terms of rhizogenesis of different cultivars of lingonberries were obtained by Gajdosová et al. (2007) 80-90 %, in supplementing the nutritional medium with 0.8 mg/l-1 IBA and 0.8 mg/l-1 activated charcoal. In order to reduce costs Gajdosová et al. (2007) root lingonberries in peat, after pretreatment in solution of 0.8 mg/l IBA.

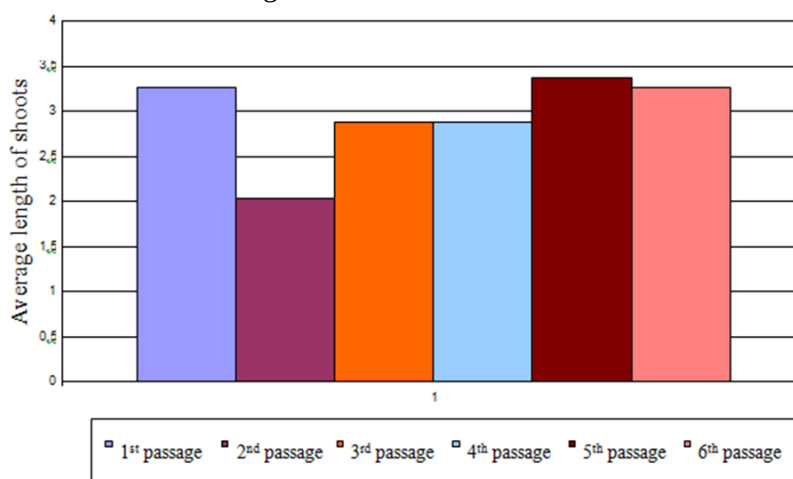


Figure 2. Average length of shoots of lingonberries (*Vaccinium vitis-idaea* L.) cv. Miss cherry

Conclusions

Lingonberries have been introduced and reproduced successfully in *in vitro* culture.

An effective system for accelerated propagation of lingonberries (*Vaccinium vitis-idaea* L.) cv. Miss cherry has been developed under *in vitro* conditions.

Suitable nutritional medium was determined based on WPM, enriched with MS vitamins and supplemented by zeatin (4 mg/l) and 2-ip (5 mg/l) for poliferation of lingonberries.

Proliferation index varied depending on passages as it varied from 1.85 (at first passage) of the sub-cultivation to 4.46 on second passage. There was a decrease in micropropagation potential after the second passage.

The greatest average length of micro shoots was registered for the fifth passage of subcultivation - 3.38 cm.

Nutritional medium WPM, with reduced salt concentration in half and enriched by 1 mg/l IAA, proved to be suitable for rooting of lingonberries (*Vaccinium vitis-idaea* L.) cv. Miss cherry, where was registered 75% rhizogenesis.

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FLORAL GROWTH OF DIFFERENT CULTIVARS OF ENNOBLED BLUEBERRY (*VACCINIUM CORYMBOSUM* L.), CULTIVATED IN TWO LOCATIONS IN KOSOVOBraha S.^{1*}, Rama P.¹, Shehaj M.¹, Gashi A.¹¹Agricultural University of Tirana, Koder -Kamëz, 1029 Tirana, Albania

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Abstract

Blueberry Flower is very attractive and quite specific, white colored into light pink in cylindrical shape. They are placed from one or from 6 to 14 flowers in a shoot. Petals are opened only in the last part, the pollen is quite fragrant with the deep drawing insects in flowers, it is heavy, so the wind does not affect pollination. Anthers are shorter than pistillate (stigma), the anthers is from 8 to 10, so that pollen can not fall in the stigma, therefore the pollinating is exclusively performed by insects. During our two years of research (2012 - 2013), it is determined the length of the petals, the diameter of the flowers near the stigma (pollen traps), the Ennobled blueberry (*Vaccinium Corymbosum* L.) in two localities with different altitudes above sea level ; Brezovicë (1043 m) and Llukë e Epërme (490 m). The researching has included six cultivars: "Duke", "Elliott", "Legacy", "Spartan", "Chandler" and "Bluecrop". The greater floral length significantly it has cultivar Chandler (1.202cm), while the smallest length possesses cultivar Spartan (0.815cm). When it comes to floral diameter also changes between larger cultivars. The greater diameter has the cultivar Duke (0.661cm), while a less value possesses cultivar Elliott (0.410cm).

Key words: flower, length of the petals, diameter of the flower, stigma, anther, pollen.

Introduction

Characteristic of blueberry flower is that the petals are opened only at the end, nectar are based as the ovary and are aromatic, they attract insects deep into flowers. The blueberry pollen is sticky and relatively heavy so the pollen can not be easily transmitted through the wind as is the case of other plants. Moreover considering the shape and position of blueberry flowers, and the position of the anthers prevents pollen falls from the tassels on the pistillate kreze even the cultivars that are self pollination. Highbush Blueberries is partly self pollination or self pollening, but nevertheless during pollination from outside (alogami) fruit size is larger and such fruits ripen earlier. Therefore blueberry flowers are firmly pollened by insects. Blueberry flowers are opened for 5-8 days. However studies show that if a blueberry flower is not pollinated for 2-3 days after opening, such flower is unlikely to connect fruit (*Sonia Schloemann*). Number of failed flowers to blueberries is very small compared with other trees. The data shows that the blueberries at the same time we have flowers and fruits which also corresponds to as a successive ripening fruit (M. Nikolić, Milivojevic J. 2010). Floral buds get damaged at -6.1 ° c and the flowers of blueberries get damaged at -3.1 ° c which is rarely anything more having in mind that

blueberries with high crown bloom quite late in the second half of May. Many cultivars are parthenocarpic or able to form fruit without pollination, however parthenocarpic fruits are smaller, ripe later and with the weaker taste. Using gibberellic acid can increase fruit size. It is very important that the cultivars pollinate with pollen from other cultivars (alogamy). It should be emphasized that there are significant differences between blueberry pollination process through bombus bee *Apis mellifera* *impatiens*, as in the yield of fruit, as well as per fruit size, e.g. during the blueberry pollination through bombus *impatiens*, fruit yield is 80.86%, while the pollination of blueberry through the bee *Apis mellifera*, fruit yield is 65.89%, so, a difference of 14.65% in yield of fruit (Alfonso Torres., Rene Ruiter 2006). The Pollinated flowers through the *impatiens* bombus, bears greatest fruit than those flowers that are pollinated by bees, also the quality of the fruit is better. According to data from the year of 2005 -2006, as far as pollinating through bombus *impatiens* has a yield increase of 39% from 2005 to 2006. While in pollinating through bees, the increase of productivity from year to year is only about 5% (Alfonso Torres., Rene Ruiter 2006). While the increase in weight of grain is of 31% for the first year and 4% in the second year (Alfonso Torres., Rene Ruiter 2006). From these data it appears that bombus *impatiens* is a highly successful pollinator of blueberries. As a result of this, blueberry growers have started to present bombus *impatiens*, as commercial colony. Bombus *impatiens* have relatively long mouth parts, which it allows them to visit the blueberry cultivars with deep nectar, because the blueberry flower is quite deep and of cylindrical shape while the other bees can not penetrate the nectar.

Materials and methods

As the material for research we have been served with six cultivars of ennobled American blueberries, *Vaccinium corymbosum* L. which were two years old. Cultivars that were involved in the experiment are: Bearing, Elliott, Legacy, Spartan, Chandler and Bluecrop. The direction of the rows in Qershiza - Brezovica and Luka Eperme is situated in north-south. Comparison of floral growth of different cultivars of blueberries, length is measured in (10 flowers per bush) and the diameter of the flower near the pistillate is also (10 flowers per bush), which means $10 \times 6 = 60$ flowers per cultivar) . We're served with the block method and have randomly selected bushes involved in the research. The data were statistically processed at the end of their collection. Measurement is performed with the help of shubler (nonius), while the values are expressed in centimeters. Each cultivar was introduced in four repetitions of six bush for each cultivar.

Results and discussion

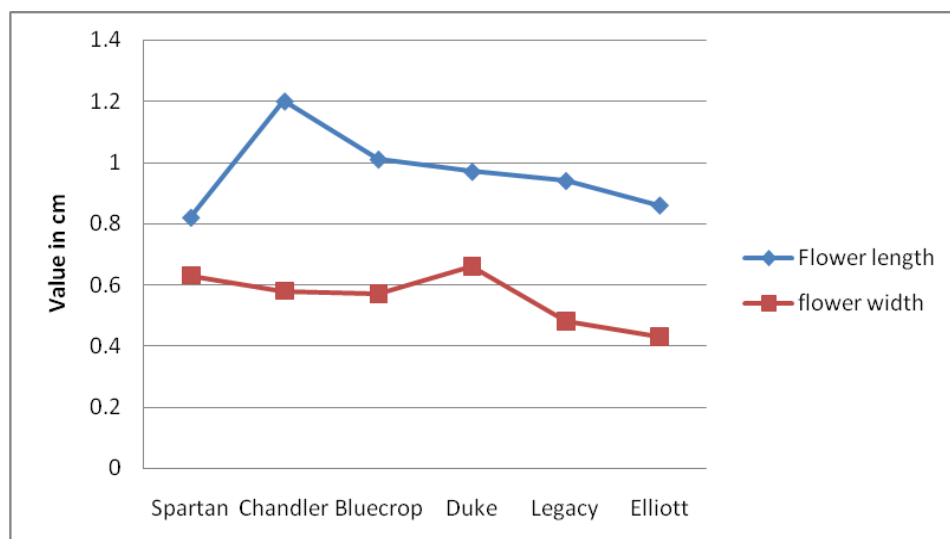


Figure 1. Average length and width of flowers of six blueberry cultivars

Table. 1

Cultivar (A)	The locality		
	Brezovicë	Llukë e Epërme	Average (A)
Spartan	0.815**	0.817	0.816 Ns
Chandler	1.202**	1.180	1.191 Ns
Bluecrop	1.015	1.006	1.010 Ns
Duke	0.973	0.960	0.966 Ns
Legacy	0.944	0.930	0.937 Ns
Elliott	0.863	0.885	0.874 Ns
Average (B)	0.968 Ns	0.959 Ns	Interaction
The factors	A	B	A x B
LSD 0.01 %	0.344	0.269	0.575
LSD 0.05 %	0.277	0.216	0.458

Legend : Ns = no significant, * = significant, ** = very significant

There are different levels of statistical differences between cultivars in terms of flower development. Greater flower length possesses cultivar Chandler (1.202 cm) and flowers with smaller length are noted by the cultivar Spartan (0.815 cm). High significant differences are observed between cultivar Chandler with cultivars Spartan and Elliot, while Chandler and Bluecrop cultivars have high significant differences between themselves as well as cultivars Duke and Legacy that have no significant differences among themselves. From this we see that there are significant differences regarding the dimensions of flowers between cultivars but there are no significant differences between cultivars at different heights above sea level. So right these differences between floral dimensions is being described as of genetic cultivars.

Conclusions

- In connection with the dimensions of the flowers for six highbush blueberry cultivars: Bluecrop, Chandler, Duke, Elliot, Legacy, and the Spartans, which have been studied over two years, 2011 -2012 is concluded that the cultivar Chandler has longer flowers (1.202 cm), while the small flowers for the length possesses cultivar Spartan (0.815 cm), compared to other cultivars. Larger floral diameter possesses cultivar Duke (0.661cm) while the smaller diameter of the flower has cultivar Elliot (0.410 cm).
- The flowers are white to reddish in cylindrical shape (like a bell). The number of fringes on the flower is from 8 to 10. The fringe are shorter than the pistillate it means are in a lower position than the pistillate.
- As in regards of the possible pollination, bearing in mind the shape and position of blueberry flowers, we conclude that the pollen of Ennobled blueberry flowers with high shrub in the easiest way is released from the tassels with the help of insects which during the search for the food make vibrations. Through the muscle vibration of wings it shakes off the pollen from the tassels. This feature owns *bombus* spp. While bees as (*Apis mellifera*) do not have this feature and this makes it more efficient compared this bee with Bumblebee.

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UDC: 634.22 (497.2)
Original scientific paper**SUSCEPTIBILITY OF GERMAN PLUM CULTIVARS TO MAIN FUNGAL DISEASES GROWN IN THE REGION OF TROYAN**Stoyanova T.^{1*}¹Research Institute of Mountain Stockbreeding and Agriculture, Troyan 5600, Bulgaria

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Abstract

The main goal of this research is to found the relation towards some fungal diseases of German plum cultivars 'Tegera', 'Hanita', 'Yo yo' and 'Elena' in agroecological conditions of Troyan (Bulgaria). Research was done during the period 2011/2014 under field conditions, at the Research Institute of Mountain Stockbreeding and Agriculture in Troyan, through several experiments. The attack of red leaf spot (*Polistigma rubrum Pers. D*), rust (*Tranzshelia pruni spinose (Pers.) Diet.*) and fungal shot hole disease (*Stigmia carpophila*) was studied. The favourable conditions for the development of diseases during the study enable us to make a comparative assessment of their susceptibility. Cultivar 'Hanita' is highly susceptible to shot hole disease and rust. Cultivars 'Yo yo', 'Tegera' and 'Elena' varied from slightly susceptible to susceptible towards the rest of the fungal diseases. There is a direct correlation of precipitation amount during the year and the level of manifestation of diseases.

Key words: plum, cultivars, fungal diseases, susceptibility

Introduction

Plum is one of the traditional species for cultivation in mountain regions in Bulgaria. Plum can give good production results at higher altitude on slopes. The main rootstock for plum is Yellow Mirabella. This rootstock has a strong root system, which can attaches itself well to the soil (poor in assimilates), which are typical for these regions. In the past, 'Kystendilska plum' was used as the main plum cultivar (Iliev et al., 1977; Iliev et al., 1985; Anzin, 1956; Vitanova et al., 2006). Despite its many positive and economic qualities and because of its strong susceptibility to economically important diseases (such as sharka and red leaf spot) its growing areas have decreased dramatically. Sharka dissemination on a large scale has led to the inability to grow sharka susceptible cultivars and to produce plums in the main plum regions. Introduction of sharka tolerant cultivars in production is one of option to solve this problem (Trifonov, 1972, 1983). The new requirements lead to enrichment of plum assortment and introduction of cultivars, which are in demand on the European market and acceptable for Troyan region.

Cultivar Stanley is the main cultivar in Bulgaria, which determines the economic significance of the different diseases and pests. Nowadays production of Stanley covering up to 85% of the plum areas. The cultivar is highly susceptible to some fungal diseases – early and late brown rot (*Monillinia laxa* and *Monillinia fructigena*), rust (*Tranzshelia*

pruni spinose (Pers.) Diet.) (Stoyanova et al., 2012, Tsonkovski, 1970). The symptoms of infection and development of diseases could be observed since early spring, during bud-formation period, flowering and fruit formation stages of plum trees. A complex of factors has an influence over their natural manifestation: time and way of infection, humidity, temperature, direction and speed of winds, age and vigour of host plants rootstock, strain variety of the cause, soil type, weeds, and microclimate in the plantation.

Growing the cultivars which are tolerant and resistant to the main economically significant diseases, allows their cultivation under reduced plant protection. The aim of the research was to study the susceptibility of new German plum cultivars ('Tegera', 'Hanita', 'Yo yo' and 'Elena') to some fungal diseases introduced in the Trojan region.

Materials and methods

The observations were conducted on German cultivars 'Tegera', 'Hanita', 'Yo yo' and 'Elena', at demonstration plum plantations, which were planted in 2001 and 2004 at light grey forest soil with a slope of 3-5°C (It is foothill part of the Balkan Mountain, where the conditions for development of fungal diseases are favourable). The trees were planted in planting pits, where were added 15 kg of manure. The area was maintained under turf mulching system. The research was conducted in the period of 2011/2014, under no irrigated conditions. No plant protection was applied.

The infestation of red leaf spot (*Polistigma rubrum Pers. D.*), rust (*Tranzshelia pruni spinose (Pers.) Diet.*) and fungal shot hole disease (*Stigmina carpophila*) was followed during research period. Cultivar 'Stanley' was used as a control. The assessment was done on samples of 200 leaves per tree, which were taken evenly from the periphery of the crown, where the infection was the strongest. Leaves were grouped in grade scales for measuring of infestation. Methodology for Study of Plant Resources in Fruit Orchard Cultivars (Nedev et al., 1979) was used for analysis.

Resistance to red leaf spot:

- 0 - immune – no infected leaves
- 1 - practically resistant – with separate small spots
- 2 – slightly susceptible – spotted leaves up to 5%
- 3 – averagely susceptible – spotted leaves up to 10%
- 4 - susceptible - spotted leaves up to 25%
- 5 - highly susceptible - spotted leaves up to 50%

Resistance to rust:

- 0 - immune – no spore on leaves
- 1 - practically resistant – with separate spore
- 2 – slightly susceptible – from 2 to 5 spore
- 3 - susceptible – spore cover 1/2 from the surface of the leaf blade
- 4 - highly susceptible – spore cover 1/2 from the surface of the leaf blade

Resistance to shot hole disease:

- 1-st degree – up to 5 spots per leaf
- 2-nd degree – up to 10 spots per leaf
- 3-rd degree – over 10 spots
- 4-th degree – over 1/4 from the leaf blade
- 5-th degree – fully scabby surface of leaves

Recently climate change has been observed, which led to change in the epidemiological feature of some of the studied diseases. The serious changes in climate impose a new concept in the selection and choice of cultivars, and to study their susceptibility to diseases. In years with more rainfall and favourable temperatures at the time of infection, the damages are stronger.

Results and discussion

Disturbances were reported in the normal development of trees for 'Hanita' cultivar from the studied cultivars; as a result from the drought during vegetation period in combination with high temperatures (Figure 1).

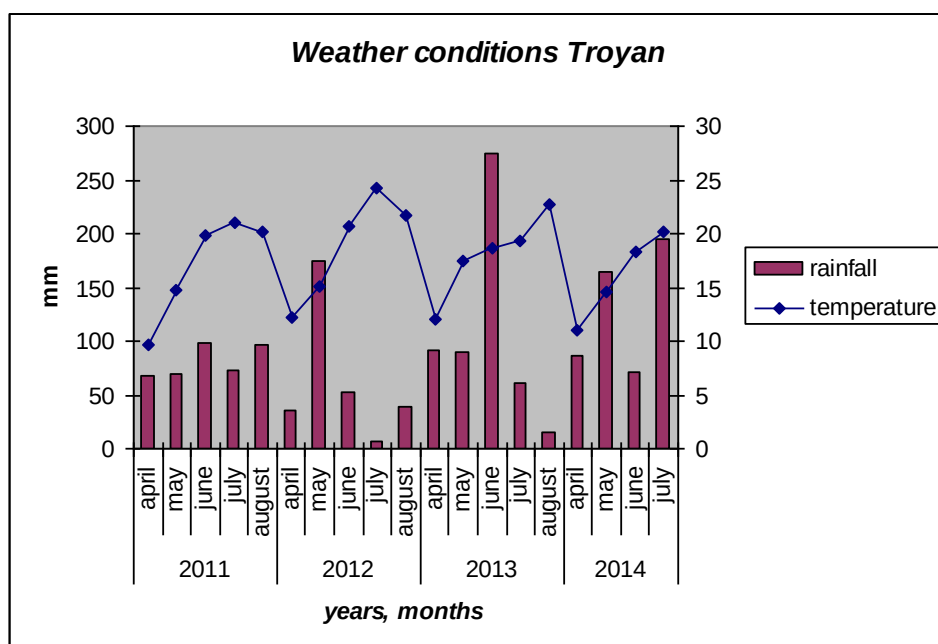


Figure 1. Climate conditions for the region of Troyan in the period 2011/2014

The unfavourable conditions resulted in fading of leaves and withering of skeletal branches. Trees remained without nutrient reserve and in the following years in combination with the favourable conditions high occurrence of rust (*Tranzshelia pruni spinose* (Pers.) Diet.) and fungal shot hole disease (*Stigmina carpophila*) was reported. In some cases the infestation index was over 50%. 'Yo yo' cultivar showed slightly susceptibility, and 'Tegera' and 'Elena' susceptibility (Table 1). The dissemination and level of fungal infestation were determined mainly by the rainfalls during time of infection. Under the climate conditions during the vegetation period in 2012, slight susceptibility to shot hole disease for 'Tegera', 'Elena' and 'Yo yo' was reported, when the rainfall amount was relatively less. Regarding the red leaf spot and rust, the cultivars showed themselves as slightly susceptible and susceptible (Table 1).

Table 1. Susceptibility of fungal diseases in plum cultivars (2011/2014)

		Hanita	Tegera	Elena	Yo-yo	Stanley
<i>Polistigma rubrum</i>	2011	5	2	2	2	1
	2012	4	4	4	2	1
	2013	4	4	2	2	1
	2014	5	2	2	2	1
<i>Stigmina carpophila</i>	2011	5	4	4	2	4
	2012	5	2	2	2	2
	2013	5	4	4	2	4
	2014	5	5	5	5	2
<i>Tranzschelia prunispinozae</i>	2011	4	3	3	3	3
	2012	3	3	3	3	3
	2013	4	3	3	4	4
	2014	4	3	4	2	4

The significant rainfall amount, in June 2013 (274.6 l/m²) during infestation period led to late infection and rust infestation in the end of summer. 'Hanita' cultivar and 'Stanley', the control cultivar, showed high susceptibility to this disease. A stronger wave of infection occurred after the prolonged rainfall periods, which were observed in 2014, when the annual rainfall amount was 1100 l/m². In combination with the favourable temperatures, epiphytic development of fungal diseases was registered for some cultivars. In addition to the accumulated infection high susceptibility to rust and shot hole disease was registered for almost all of the cultivars. 'Yo yo' cultivar only showed slight susceptibility to rust (*Tranzshelia pruni spinose* (Pers.) Diet.). Regarding the infestation of red leaf spot (*Polistigma rubrum* Pers. D.), the cultivars were slightly susceptible with the exception of 'Hanita' (Table 1). The conducted studies showed that the introduced German plum cultivars, which are grown in the Troyan region, showed diverse behaviour in relation to resistance to fungal diseases. During the study period, a tendency for stronger manifestation of shot hole disease (*Stigmina carpophila*) and rust (*Tranzshelia pruni spinose* (Pers.) Diet.) was observed.

Conclusions

The susceptibility to main fungal disease, in four German plum cultivars, has been studied, which have been grown in the Troyan region (Bulgaria). 'Hanita' cultivar shows highly susceptibility to shot hole disease and rust. 'Yo yo', 'Tegera' and 'Elena' were slightly susceptible to red leaf spot (*Polistigma rubrum* Pers. D.). They were from slightly susceptible to susceptible in relation to the rest of the fungal diseases. A direct correlation was noticed between the rainfall and humidity during the period of infection and the damage degree caused by diseases.

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**BEHAVIOR OF BARTLET AND RED BARTLET PEAR CULTIVARS
GROWN UNDER ANTI HAIL AND UV PROTECTIVE NET**Gjamovski V.^{1*}, Kiprijanovski M.², Arsov T.²¹Institute of Agriculture, University of Ss. Cyril and Methodius, R. Macedonia²Faculty of Agricultural Sciences and Food, University of Ss. "Cyril and Methodius", R. Macedonia

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Abstract

Usages of UV protective nets are one of the many practices for adapting and improve fruit production to the climate factors. The effects of UV nets on the protection of the trees from hail, sunburns of leafs and fruits, temperature of leafs and fruit as well and fruit quality were evaluated over three seasons (from 2013 to 2015) at the pear experimental orchard located in Kumanovo. Also vegetative growth productivity and yield efficiency were evaluated. The experimental orchard was established in 2010, with a planting distance 3.2 x 1.3 m. The experiment was comprised in two treatments: covered and uncovered trees. From each variant 30 trees in three repetitions were investigated. Evaluation was carried out on 2 pear varieties: Bartlet and Max Red Bartlet grafted on quince BA 29 using interstock "Gelert". The results shown higher growth on covered trees compared to uncover as a result from improvement of microclimatic condition in the orchard. Also, higher productivity of the covered trees was registered. At both evaluated varieties UV protective net demonstrated its efficiency for fruit protection against hail, sunburns, reduction of leaf and fruit temperature as well and in the quality of the fruits.

Key words: orchard, hail net, protective, productivity, fruit quality

Introduction

Implementation of net protection is gaining popularity in modern fruit production around the world to protect orchards against excessive solar radiation damage, hailstorms and flying pests (Blanke, 2007). The use of anti-hail nets is an active defense against hailstorms and the covering of orchards is becoming more and more a cost-effective technique (Torggler, 1998; Borin and Saoncella, 2000; Poldervaart, 2006). Although a number of studies have focused on the influence of hail nets on orchard ecology, (Kon et al., 1989; Ruegg, 1997), fruit quality (Widmer, 2001; Guerrero et al., 2002; Middleton and McWaters, 2002; Stampar et al., 2002), tree physiology (Blanke and Weidenfeld, 1997; Ebert and Casierra, 2000; Casierra-Posada and Ludders, 2001), spraying techniques and irrigation (Orts, 2003; Garcin and Zavagli, 2006), plant diseases (Liesenborghs, 1998), and pollination (Vaissiere et al., 2000). Mostly of them are focused on apple and peach plantation. Researches of influence of UV protective net in pear orchard are minor and far more from complete. After damage from severe hail storms and frequent occurrence of sunburn on fruits in recent years the covering of orchards with UV hail protection nets has

become an important issue for many fruit growers. Hail netting offers good protection but also entails high investment costs.

On the other hand, before the economic transformations in Macedonia pear production reached over 20.000 tones. Later, the area under orchards and pear production decreased rapidly. Nowadays, the pear production is not satisfied, even for domestic needs. During the autumn and winter period the markets are supply with imported high priced pears nearly unaffordable for the domestic consumers. The reasons for this situation lay mostly in the appearance of epiphytotic measures of the psylla and Fire Blight, for which the producers have lack of information and knowledge (Ristevski, 2004).

In the present years, interest among the producers for establishing the pear orchards is increasing. One of the manners for advancement of the production is improvement of the assortment structure and growing technologies. However, up to date only one pear orchard was established under anti hail and UV protective net. Increasingly, growers must aim to produce both higher quality and greater quantity of fruit to cover their overhead costs and turn their orchard into a successful investment as quickly as possible. These can achieved clearly, by implement modern and intensive fruit growing technologies.

This work was conducted to evaluate yield and fruit quality of 'Bartlet' and 'Red Bartlet' pears grafted on quince rootstock MA growing under white protection net.

Material and methods

The trail was carried out in pear orchard located in Kumanovo region (Northeastern Macedonia). The agro-ecological condition indicate that this area is semiarid, with annually temperature of 12.5 °C, average summer temperature of 22.3 °C and absolutely maximum of 41.5 °C. Average annually rainfalls in past ten years were 550 mm. The orchard consisted of 5-year-old 'Bartlet' and 'Red Bartlet' pear trees on quince rootstock MA. Pear cultivar Beurre Hardy used as interstock, raised as slender spindles, with a spacing of 3.2m×1.3m. Net cover system was installed in the third year of study.

For each cultivar, the variants consisted of uncovered/control trees, and trees covered with white net (with aperture size of 4 mm × 7 mm). Covered trees remained uncovered during winter (to avoid the damage to the supporting structure by the snow) until the end of flowering (to permit an efficient insect pollination in the orchard). The experiment followed a completely randomized block design, with four replications (corresponding to trees) for both cultivars. Evaluation of the effects of anti-hail nets to change of microclimate conditions, growth of the trees, productivity and quality of the fruits were conducted each year. The dynamic of the diameter growth of the trunk was followed each year. The diameter of the trees was measured during harvest season. Trunk cross sectional area (TCSA) was calculated from those measurements. Yield per tree and cumulative yield per tree were computed from the harvest data. Yield efficiency was calculated as kg/cm². The fruit quality has been determined thought weight and chemical characteristics (total soluble solids and titrate acids). In general, the analyzed fruits were sampled during the first commercial harvest. Fruit from bought variety and variants was randomly harvested from 20 different trees, 30 representative fruits were processed for all the analysis. Fruit weight was measured using digital balance. Total soluble solid (TSS) content (°Brix) was determined using a refractometer. Titratable acidity (TA) was determined on three juice samples, diluted in distilled water, and micro titrated with NaOH 0.1 N (Daza et al., 2008).

Statistical analysis was performed using SPSS software. Independent samples t-test were used to analyse all the experimental data. Results were expressed at the $P < 0.05$ level of significance.

Results and discussion

The diameter of the trunk is an integral indicator of the whole vegetative potential of the trees. The lowest mean values for TCSA at the end of 2015 were observed at covered trees from cv. 'Red Bartlet' (Table 1). Testing the variants no statistical differences were recorded. Slightly higher trunk growth was noticed on covered trees compared to the uncovered. This phenomenon probably is the result of the shading by nets. This causes significant reduction of radiations, evapotranspiration, humidity increasing and stress decreasing and positively affects the trees growth. Similar results were presented by Iglesias and Alegre (2006). The greater humidity under the nets leads to decreased plant water stress and better vegetative growth of the trees. On the average, for both of the varieties at the end of the research period, covered trees had slightly bigger TCSA (28.11 cm^2) compared to uncovered trees (27.67 cm^2).

Table 1. Trunk cross-sectional area (TCSA), cm^2

Variety	Status of the orchard	TCSA, cm^2		
		2013	2014	2015
'Red Bartlet'	Uncovered	18.84	23.69	28.51
	Covered	18.22	23.99	29.68
<i>p-value</i>		<i>0.680</i>	<i>0.807</i>	<i>0.782</i>
'Bartlet'	Uncovered	18.66	23.46	26.82
	Covered	19.81	22.91	26.93
<i>p-value</i>		<i>0.661</i>	<i>0.556</i>	<i>0.971</i>
Average	Uncovered	18.75	23.58	27.67
	Covered	19.02	23.45	28.21

The number and size of fruits determine yield. They each are in inverse proportion, so in modern fruit production seeks to provide optimal number of fruits per tree to provide optimal yield with desirable quality of the fruits (Kiprijanovski et al., 2009). The highest number per tree was obtained in 2015 (Table 2). The highest cumulative yield was observed at covered trees from bought varieties. Comparing variants statistical significant higher yield on covered at bought varieties was observed in 2014 and 2015 as well in the cumulative yield. This is due to the fact that in 2014 severe hail occurrence was registered. Hailstorm followed by strong wind happened in period when the fruits were in their final stage of growth and were very sensitive to wind and hail and could be stripped from the trees. According to Dods et. al., 2014 hail storm that appear close to harvest is more serious than a hail storm which occurs earlier in the season. Lower registered yield per tree in 2015 on uncovered trees is result for the indirect damages by hail in previous year. According to Ristevski (1995) the indirect damages reflect negatively on the productive potential for the next year through wounds on the branches and decreasing the leaf area. As a result of the decreased photosynthesis activity and spending of assimilates for wounds healing in the differentiation period, the number of floral buds per tree is decreasing.

SECTION 7. FRUIT GROWING

Table 2. Productivity of the trees

Variety	Status of the orchard	Number of fruits per tree				Yield per tree, kg			
		2013	2014	2015	Cum.	2013	2014	2015	Cum.
‘Red Bartlet’	Uncovered	39.1	19.3	29.5	87.9	8.2	4.4	7.6	20.2
	Covered	41.9	35.1	54.4	131.4	8.8	9.3	12.9	31.0
p-value		0.458	0.003	0.006	0.009	0.725	0.001	0.003	0.011
‘Bartlet’	Uncovered	57.5	31.7	50.8	140.0	11.1	7.5	13.1	31.7
	Covered	59.3	50.9	78.4	188.6	12.2	14.6	18.8	45.5
p-value		0.692	0.018	0.022	0.023	0.712	0.016	0.042	0.038
Average	Uncovered	48.3	25.5	39.8	113.5	9.6	6.0	10.4	25.9
	Covered	50.6	43.0	66.4	160.0	10.5	11.9	15.8	38.3

The yield efficiency is a complex index which includes the vegetative growth of the tree and its productivity (Uselis, 2006). The best yield efficiency (Yield/TCSA) was found on covered trees from bought variety in average 2.29 kg/cm². In contrary, the lowest yield efficiency (Yield/TCSA) was found on uncovered trees (Table 3). This is probably due to their reduction of yield caused by hail occurrence.

Table 3. Yield efficiency, kg/cm²

Variety	Status of the orchard	Yield/TCSA kg/cm ²			
		2013	2014	2015	Average
‘Red Bartlet’	Uncovered	2.08	0.81	1.03	1.31
	Covered	2.30	1.46	1.83	1.87
‘Bartlet’	Uncovered	3.08	1.35	1.89	2.11
	Covered	2.99	2.22	2.91	2.71
Average	Uncovered	2.58	1.08	1.46	1.71
	Covered	2.65	1.84	2.37	2.29

Appearance and quality of the physiological mature fruits is important factor for realizing the marketable fruits. Fruit weight, the content of soluble solids and total titratable acids depend on the variety and the conditions of the environment. According to Hudina et al. (2001), the applied agrotechnical and pomotechnical measures such as fertilization, irrigation and the training system can influence these parameters. Analyzing the parameter for the fruit quality, insignificant differences are noticed between evaluated variants. Covered trees in average have slightly bigger average fruit weight (Table 4). Lower fruit weight on covered trees was registered at cv. ‘Red Bartlet’ (209.5 g) compared to those grown uncovered (210.1 g). On colored pear variety (‘Red Bartlet’) lower coloration of the fruits at covered trees was notice. Higher amount of total soluble solids (15.3 °BRIX in average) and lower amount of total acids (0.29% in average) was measured on covered trees compared with uncovered.

Table 4. Quality characteristics of the fruits

Variety	Status of the orchard	Fruit weight, g	Total soluble solids, °BRIX	Total acids, %
‘Red Bartlet’	Uncovered	210.1	13.9	0.33
	Covered	209.5	14.3	0.30
‘Bartlet’	Uncovered	192.5	16.3	0.28
	Covered	205.5	16.2	0.27
Average	Uncovered	206.3	15.1	0.31
	Covered	207.5	15.3	0.29

Conclusion

The research showed that UV protective nets have big impact in pear orchards protection in case of hail. The damages caused by hail and strong wind which is a following element in cases like this, provoke different physiological disorders at the trees on bought varieties, which eventually reflects negatively on the productive effects for at least two productive cycles. The influence of that UV protective net on vegetative growth of the trees and fruit quality is insignificant. The fruits from covered trees have slightly higher fruit weight compared with those from uncovered trees. The research shown, that the influence of the UV protective nets towards the concentration of soluble solids and total titratable acids is minimal. The fruits from the covered trees the higher content of soluble solids and lower concentration of total acids respective to the ones of uncovered trees. Although setting up UV protective nets is an additional investment, in modern fruit production UV protective represent the most effective way for so-called safe production with minimum risk.

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THE INFLUENCE OF SOME PHYTOHORMONES ON THE ROOTING OF PLUM CUTTINGS

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Abstract

The purpose of the research was to determine the influence of some phytohormones on the rooting of plum hardwood cuttings. The research was performed with cuttings of the plum St Julien (*Prunus insititia*), which is used as a semi vigorous vegetative rootstock for several stone fruits. The research was conducted in a commercial nursery located in Argulica, Stip. For the research were used two phytohormones: indolebuteryc acid (IBA) and 1-Naphthaleneacetic acid (NAA). A combination of both was also used – in powder and liquid form, in different concentrations. From each branch were obtained two types of cuttings, one from the base and one from the middle. In the end of the vegetation period the following characteristics were evaluated: the percentage of rooting, the diameter of the cuttings, the diameter and the length of the shoots, the mass, the length and the quantity of roots. On the basis of our research we determined that the cuttings taken from the base of the branches have bigger percentage of rooting in comparison with those from the top. The cuttings treated with 2% IBA had the biggest diameter. The cuttings treated with 0,1% IBA had the biggest length and number of roots. The cuttings treated with 2% IBA had the biggest average mass of the roots while those treated with 0,1% IBA had the biggest length of the roots.

Key words: St. Julien (*Prunus insititia*), cutting, rooting.

Introduction

A successful fruit production, apart from the choice of fruit species, variety, suitable soil and climate conditions, agro-technology and pomological actions, also largely depends on the choice of rootstock. The rootstocks are plants that can be propagated from seed or with the use of vegetative reproduction, so that we can graft and get grafted fruit trees (Kolekevski et al., 2004). When propagated vegetative, the rootstocks keep the morphological and physiological characteristics of the parent plant. Some important biological, physiological and economical characteristics of these rootstocks are: they receive all of the morphological and physiological characteristics from their parent plant, they are uniform and homogenous in all of the characteristics, they have smaller vigor, and they grow and start bearing faster. The essence of propagation by cuttings consists of the following: one part is separated from the parent plant, the lower part of it is put in the soil and the top remains above the ground (Kolekevski et al., 2004). From the adventitious

buds in the soil adventitious roots arise, and from the vegetative buds above the ground are formed shoots. The accelerated production of planting material with hardwood and softwood cuttings is mostly used when propagating fruit trees of deficient fruit species used in the production or for a genetic selection or when there are not enough plants for propagation. The cuttings from the annual growth are rich in meristematic tissue, especially hardwood cuttings, which have enough plastic materials needed for good rooting (Spirovska, 1982). According to Jovanović (1976), phytohormones are mostly used for strengthening and improving of the root formation on the cuttings. Their application is due to the stimulating effect they have on the cuttings with a natural rooting ability by forming more and better developed roots. Especially significant results have been achieved with the use of phytohormones from the auxin family (IBA, NAA). According to Kefford and Goldacre (1961) a successful rooting of cuttings from perennial plants with the use of IBA and NAA is due to the effect of exogenous phytohormonal chemicals that direct the metabolic processes in the direction of growth and development of cells or certain organs. According to Kapetanović and (1974), the rooting process depends on the time when the cuttings are made. The cuttings made in January or February are rooting better than those made in November or December. In all plant species there is a certain range of type and concentration of auxins that either stimulate or slow down the growth (Jovanović, 1976). Theoretically, nowadays there are studies regarding the regulatory role of phytohormones in the process of root formation. In practice, many efforts are made in order to find optimal conditions for rooting the cuttings of different species, especially where the root formation is hard (Stefančić et al., 2007).

The plum St. Julien originates from England (East Malling). It originates from a collection of seedlings which belong to the species *Prunus insititia* L. It can propagate with hardwood cuttings and has a rooting ability. It has a good affinity with almost all plum varieties, and it can be used as a rootstock for other stone fruits (peach, nectarine, apricot). Fruit trees with St. Julien as a rootstock have moderate vigor and start bearing 3 – 4 years after the planting. The purpose of our research was to examine the rooting of the St. Julien cuttings while using IBA and NAA phytohormones.

Materials and methods

The cuttings were obtained from a nursery in Argulica which is established with certified stock plants with origin from Netherlands. The cuttings were collected in January. They were kept in a plastic bag in a refrigerator on a temperature of 2°C until the end of February. Two types of cuttings have been obtained from each branch – one from the base and one from the middle. The bottom part of the cutting is cut flat under the node and the top slightly obliquely in the opposite direction of the bud. Then the cuttings were treated with individual phytohormones and with a combination of them in different concentration in the following variants:

1	IBA 0,1% dipped 1 minute	Base part
2	IBA 2% powder	Base part
3	IBA 0,1% + NAA 0,1% dipped 1 minute	Base part
4	NAA 0,1% dipped 1 minute	Base part
5	IBA 0,1% dipped 1 minute	Middle part
6	IBA 2% powder	Middle part
7	IBA 0,1% + NAA 0,1% dipped 1 minute	Middle part
8	NAA 0,1% 1 minute	Middle part
9	Control (not treated)	Base and middle part

After the treatment the cuttings were put in black plastic bags and were kept in a dark room on room temperature for a period of 30 days. After this period the cuttings were planted in a nursery at a distance of 10 cm inside the row and 100 cm between the rows, on a light soil. For the research 30 cuttings were used for each variant, divided by 3 repetitions for each variant. During the vegetative period, the nursery was grown using standard technology. At the end of the vegetative period the cuttings were dug out after the leaves have dropped and the following parameters were determined:

- Number of received (rooted) cuttings
- The diameter of the cuttings
- The diameter and length of the shoots
- The mass, length and number of roots by cuttings.

The percentage of received cuttings was determined according the number of received cuttings and the total number of cuttings set. The diameter of the cuttings and the shoots was measured with a caliper. The length of the shoots and the roots was measured with a measuring tape. The mass of the roots was determined with scale (KERN EMB 600-2, d=0,01g).

Statistical analyses were performed using SPSS (IBM SPSS Statistics 19). The differences were evaluated by anova analysis through General linear model procedure. After the GLM analyses, post hoc comparison of the means were calculated by LSD. Results were expressed at the $p < 0.05$ level of significance.

The statistical analysis for type of cuttings was performed by an analysis of the variance and the testing of the differences by a T-test.

Results and discussion

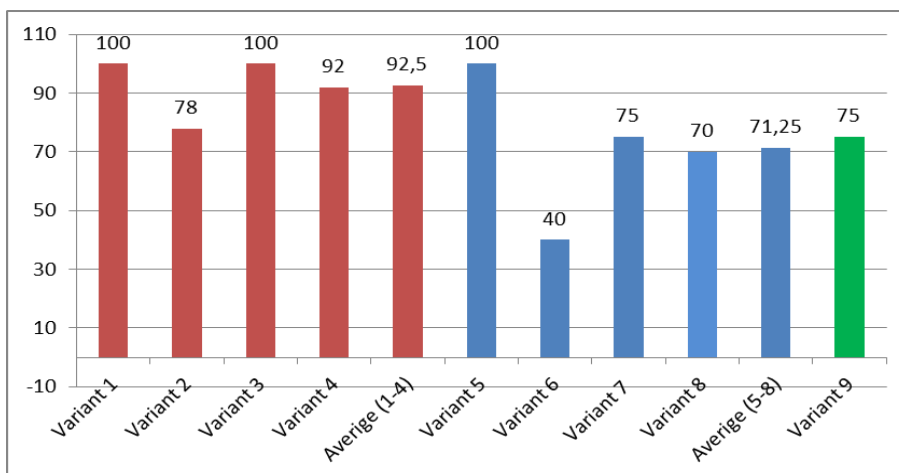


Figure 1. Percentage of rooting

In figure.1 it is shown that the variants 1, 3, and 5 have a maximum percentage of rooting, and the lowest percentage was found in variant 4. If we observe the average rooting percentage of the cuttings depending if they are taken from the base or from the top of the branch we can see that there is a rooting percentage of 92,5 in the cuttings taken from the base part in comparison with the cuttings taken from the top which have rooting percentage of 71,25.

Table 1. Morphometric characteristics of the cuttings according the variants

Var.	Diameter of the cuttings (cm)	Diameter of the shoots (cm)	Length of the shoots (cm)	Number of roots by cutting	Mass of the roots by cutting (g)	Total length of the roots (cm)
1	11,04	8,3	119,57	27,29 ^a	3,42 ^{bcd}	289,67 ^a
2	11,63	9,56	116,71	19,86 ^{bcd}	4,57 ^{ab}	239,33 ^{af}
3	11,56	9,26	101,29	26,71 ^a	4,19 ^{ad}	278,33 ^{ac}
4	11,41	7,96	86,14	17,71 ^{cd}	4,39 ^{ac}	181,67 ^{def}
5	11,37	9,39	121,14	24,57 ^{ab}	3,47 ^{bcd}	383,67
6	10,27	7,68	84	17,11 ^{cd}	5,46 ^a	189,33 ^{bcd}
7	10,29	9,41	93,57	22,86 ^{ac}	4,15 ^{ae}	281,67 ^{ab}
8	10,64	8,6	97,29	22,71 ^{ac}	3,6 ^{bcd}	261 ^{ad}
9	9,04	8	87,88	15,75 ^d	3,36 ^{bcd}	258,5 ^{ae}

*Values followed by the same letter in the column were not statistically different.

In table 1 we can see that the diameter of the cuttings and the shoots from all variants fulfills the recommended thickness for grafting which is between 8 and 12 mm. Following data from the table 1 it can be notice that higher number of roots per cutting was obtained at variant 1 followed by variant 3 with statistical differences to variant 2, 4, 6 and 9. The lowest number of roots per cutting was registered at control variant 9 but without statistical differences with variant 2, 4 and 6.

The highest mass of the roots was measured in variant 6 with statistical differences with variants 1, 5, 8 and 9. The lowest mass was registered at the control variant without statistical differences with all of the variants except variant 6.

The longest length of roots was obtained at variant 5 with statistical difference to all of variants. The shortest length was measured in variant 4 without statistical differences to variants 2, 6, 8, and 9.

Table 2. Types of cuttings

Type of cuttings	Diameter of the cuttings (cm)	Diameter of the shoots (cm)	Length of the shoots (cm)	Number of roots by cutting	Mass of the roots by cutting (g)	Total length of the roots (cm)
Base part	11,41	8,77	105,93	22,89	4,14	247,25
Middle part	10,64	8,77	99	21,81	4,17	278,92
P - Value				0.124	0.734	0.339

According to the independent samples t - test between the means from the base and middle part of the cuttings, no statistical significant difference was found, which means that we can use cuttings from the base and middle parts of the branch but only if they are uniformly developed.

Table 3. Morphometric characteristics of the treatments

Treatments	Diameter of the cuttings (cm)	Diameter of the shoots (cm)	Length of the shoots (cm)	Number of roots by cutting	Mass of the roots by cutting (g)	Total length of the roots (cm)
IBA 0,1%1min.	11,21	8,85	120,36	25,93 ^a	3,45 ^a	336,67 ^a
IBA 2% powder	10,95	8,62	100,36	18,49 ^b	5,02 ^b	214,33 ^b
IBA 0,1% + NAA 0,1% 1 min.	10,93	9,34	97,43	24,79 ^a	4,17 ^{ab}	280 ^{ab}
NAA 0,1% 1 min.	11,03	8,28	91,72	20,21 ^b	4,00 ^{ab}	221,34 ^b
Control	9,04	8	87,88	15,75 ^b	3,36 ^a	258,5 ^{ab}

*Values followed by the same letter in the column were not statistically different.

Concerning the type of treatments our research shows that high quality rootstock can be reached by using IBA 0,1% 1min solution. The treatment IBA 2% powder treatment has the biggest mass of the roots with statistical significant difference with the treatments IBA 0, 1% 1min and the control. The treatment IBA 0,1% has the longest total length of the roots with statistical significant difference with the treatments IBA 2% powder and NAA 0,1% 1min (table 3).

Conclusion

On the basis of our research we can make several conclusions:

The cuttings taken from the base part of the branch have a higher percentage of rooting (92.5) in comparison with those taken from to top part (71.25) which is also satisfactory.

The diameter of the cuttings and the shoots meets the recommended size for grafting.

The highest (100%) percentage of rooting was observed at the following variants: IBA 0,1% dipped 1 minute / base part, IBA 0,1% + NAA 0,1% dipped 1 minute / base part and IBA 0,1% dipped 1 minute / middle part.

The results showed that the best treatment for rooting of plum St Julien (*Prunus insititia*) cuttings is IBA 0,1% dipped 1 minute.

We can use cuttings of the plum St Julien (*Prunus insititia*) from the base and from the middle part of the branch but only if they are uniformly developed.

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**MODELLING THE BEARING POTENTIAL OF BLACKBERRY
(*Rubus fruticosus*) cv. 'THORNFREE'**Cvetković M.^{1*}, Mičić N.¹, Marušić M.¹, Bosančić B.¹¹University of Banja Luka, Faculty of Agriculture

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Abstract

Modern production of semi-erect blackberry cultivars is mostly practised under trellis systems using consistent cane management techniques when selecting primocanes for fruiting, and cane pruning operations. Selecting the optimal number of primocanes per plant is designed to assure high yields of blackberry fruit of satisfactory quality under given production conditions. A study was conducted to determine the effect of cane density on the yield potential of blackberry (*Rubus fruticosus* L.) cultivar 'Thornfree' in the region of the Municipality of Doboj (Bosnia and Herzegovina). Yield potential characteristics of the cultivar under a load of two, three and four primocanes per plant were analysed. The number of primocanes per plant affects the degree of lateral branching and the number and position of inflorescences. The highest yield during the research period was obtained in blackberries with three canes per plant (30.86 t/ha) and the lowest in two-cane plants (20.02 t/ha). Fruit size (weight, length and width) was the highest in plants with two canes. Given the satisfactory fruit quality and total yield, the load of three trainable primocanes per plant in 'Thornfree' can be considered optimal for given production conditions.

Key words: *inflorescence, fruit, yield***Introduction**

The largest blackberry production regions are Mexico, Europe (Serbia) and the United States of America (Oregon) (Strik and Finn, 2012). Milošević(2000) reports that blackberry production in Serbia has been on the rise primarily due to the good price on the world market and the expansion of production of 'Čačanska Bestrna' ('Čačak Thornless'). 'Thornfree', as a leading cultivar in Serbia (Strik et al., 2008) and Bosnia and Herzegovina (Petrović et al., 2002), is a semi-erect cultivar (Mišić and Nikolić, 2003, Šoškić, 1984) typically grown at a spacing of 1.0 - 1.8 × 3.0 - 3.6 and ensuring yields of 20.00 to 30.00 t/ha. Strik and Fin (2012) described that semi-erect cultivars such as 'Thornfree' are generally grown on a multiple-wire trellis, although some producers also use the V-shaped trellis system. Blackberry primocanes are commonly pinched at a height of 0.45 m to 1.8 m to promote lateral branching and, hence, increase yield per unit area. Cane density can very often affect the development of lateral branches and numbers of inflorescences (Lučić et al., 1986), thus potentially affecting total yield (Cortell and Strik, 1997). Increasing the number of primocanes per plant decreases the average number of lateral branches, indicating that it leads to no yield increases (Takeda et al., 2003). Sufficient numbers of blackberry primocanes per plant should be retained immediately

upon their formation, while the rest should be removed (*Mićić, 1989*). The realization of high yields in blackberries is possible under the consistent use of cane management practices, involving shortening primocanes to the uppermost wire during the growing season, by cutting back lateral branches to 6 to 8 buds, which are correctively pruned back to 3 to 4 buds just before the onset of the following growing season (*Mićić and Cvetković, 2005*). The objective of this study was to determine the effect of different cane densities under regular cropping conditions on the fruiting of blackberry cultivar 'Thornfree'. Also, the research focused on differences in fruit quality depending on cane density and harvest dynamics.

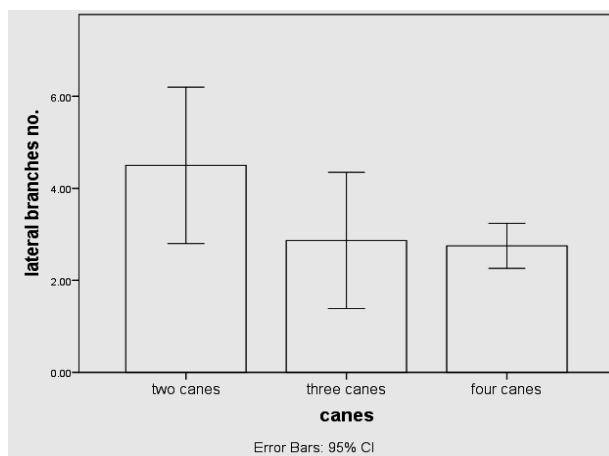
Material and method

The study on the effect of cane density on the yield potential of 'Thornfree' blackberry (*Rubus fruticosus* L.) was conducted in a blackberry planting owned by Miroslava Marušić, B. Sc. in Pomology and Viticulture. The planting is located at Gornja Paklenica – Municipality of Doboj (44°37'15.76"N, 18°09'30.63"E), at an elevation of 450 m. The planting was established in 2002 on a pseudogley soil having fair to poor productivity properties and a pH of below 5.0. The location was well supplied with precipitation, with about 944.0 l/m² during the year, of which 514.0 l/m² during the growing season (period 1981-2010, *RHMZ, 2013*). The average annual air temperature was 11.4°C. The planting was trained to a trellis system at a spacing of 3 x 1.5 m. The trellis consisted of wooden posts and four rows of galvanised wire (first wire at 50 cm above ground level, the other three rows of wire spaced 40 cm apart). Cultural practices were used regularly in the planting. The experiment was established in 2011 in two-, three- and four-primocane retention treatments, with five replications per treatment. The cane management practices in 2011 (regardless of number of primocanes per plant) involved shortening primocanes to the uppermost wire (primocane height about 2.0 m). After topping, the resulting lateral branches were cut back to 6 to 8 buds during September and subsequently correctively pruned back to 2 to 3 buds before the onset of the growing season (*Mićić and Cvetković, 2005*). Fruiting parameters were examined in 2012. The following fruiting parameters were observed on canes during the growing season: number of lateral branches on the canes, number of inflorescences formed on the lateral branches, number of inflorescences formed from axillary mixed buds, number of fruits developed on the inflorescences formed on the lateral branches, number of fruits developed on the inflorescences formed from the axillary mixed buds. In the second part of the growing season, during the harvest phenophase (at five successive picking dates), physiologically mature blackberry fruits were characterised for fruit weight, fruit length and fruit width. Statistical analysis was carried out via the statistical software package SPSS 22 (IBM 2013).

Results and discussion

The reduction in primocane numbers per plant is compensated through increases in the number of lateral branches formed on the canes (Graph1), which is, *inter alia*, dependent upon the cane management practices used in the previous season (*Mićić and Cvetković, 2005*) which ensure the development of lateral branches. The tested treatments showed statistically significant differences in the number of lateral branches ($F=4.16$, $p=0.042$)

between two-primocane plants and three- and four-primocane plants, while no significant difference was observed between three- and four-primocane plants.



Graph 1. Number of lateral branches on the cane depending on the number of canes per plant

In their study of ‘ChesterThornless’ blackberry plants that developed 2, 4 and 6 primocanes, *Takeda et al.* (2003) found that increasing the number of canes per plant led to a decreased number of lateral branches, eventually resulting in no significant yield increase, regardless of the number of primocanes per plant. In all tested combinations, more inflorescences were developed directly from the axillary mixed buds on the cane than on the lateral branches (Table 1).

Table 1. Average and total numbers of inflorescences developed on lateral branches and on the cane

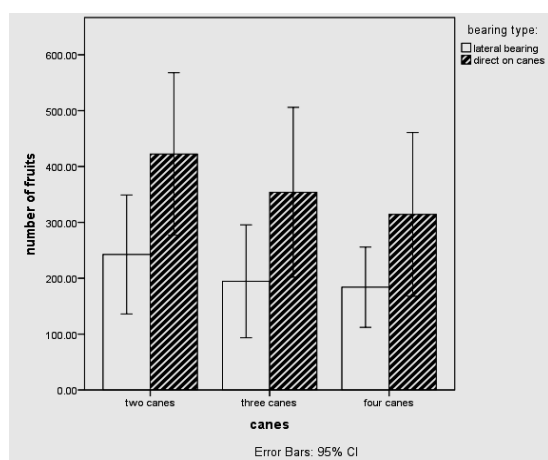
	Two canes	Three canes	Four canes
Average number of inflorescences per lateral branch	2.8 ± 0.124	2.9 ± 0.148	3.2 ± 0.114
Total number of inflorescences on lateral branches	13.0 ± 2.030	8.5 ± 1.214	8.7 ± 0.696
Total number of inflorescences that developed from axillary mixed buds	16.0 ± 1.333	18.5 ± 0.729	18.0 ± 0.390

Evidently, plants that developed two trainable primocanes with more lateral branches formed their yield potential on the inflorescences formed right in these positions. *Takeda et al.* (2003) reported that the number of inflorescences (regardless of position) in ‘ChesterThornless’ blackberry was 16.0 on plants with 6 floricanes, 17.5 on plants pruned to 4 floricanes and 23.2 on plants with 2 floricanes. The number of canes per plant has no significant effect on the number of fruits formed on inflorescences (Table 2).

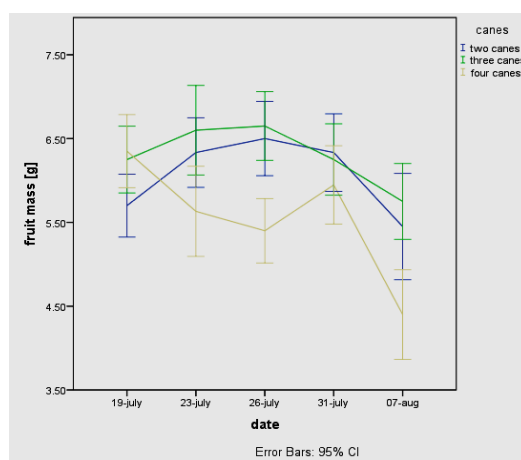
Table 2. Average number of fruits on inflorescences developed on the lateral branches and on the cane

	Two canes	Three canes	Four canes
Average number of fruits on inflorescences developed on lateral branches	24.8 ± 0.729	29.1 ± 1.153	23.9 ± 0.614
Average number of fruits on inflorescences developed from axillary mixed buds on the cane	26.2 ± 0.881	26.8 ± 0.690	21.2 ± 0.326

The total number of fruits on inflorescences (Graph 2) shows that statistically significantly more fruits ($F=18.396$, $p<0.000$) were determined on the inflorescences formed from axillary mixed buds on the cane than on those formed from buds on lateral branches. Results show that the number of canes per plant had no effect on the number of fruits on inflorescences. Fruit weight was affected by both cane number per plant and harvest date (Graph 3), given the highly significant interaction between the two factors ($F=3.22$, $p=0.002$), as determined by the analysis of variance.

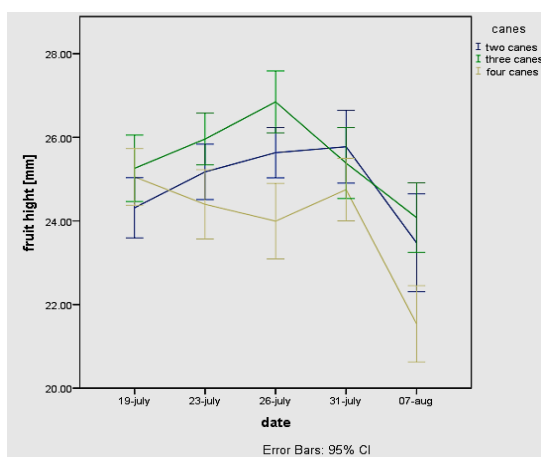


Graph 2. Number of fruits per inflorescence

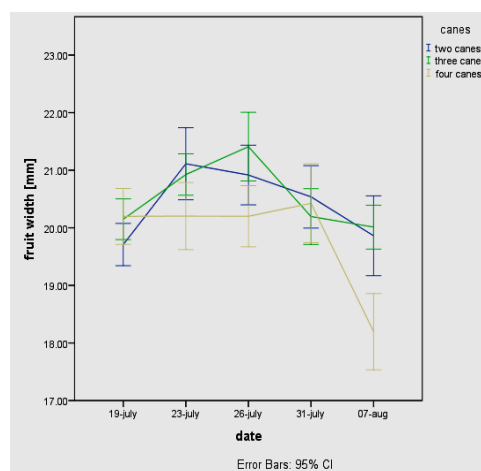


Graph 3. Average fruit weight depending on number of canes per plant and harvest date

At the beginning of harvest, the lowest fruit weight was obtained in four-cane plants, and this trend continued throughout the harvest period. A statistically significant difference was observed in fruit weight between plants with 2 and 3 canes and those with 4 canes, at all harvest dates except on 31 July when the difference, although present, was not statistically significant. *Takeda et al.* (2003) reported similar data on fruit weight in 'ChesterThornless', the highest in plants with two floricanes, compared to plants with 4 and 6 floricanes, with the difference being statistically significant. The difference in fruit weight between plants with 2 and 3 retention canes was not statistically significant. A similar pattern was observed in fruit length ($F=3.13$, $p=0.002$ – Graph 4) and fruit width ($F=3.88$, $p<0.000$ – Graph 5).



Graph 4. Average fruit length



Graph 5. Average fruit width

The analysis of average yields depending on cane number per plant (Table 3) reveals that 'Thornfree' yield ranged from 20.2 to 30.86 t/ha (Strik *et al.*, 2008), but was not directly correlated with cane number per plant, which is in agreement with other studies on the effect of cane density on the yield of blackberry cultivars (Cortell and Strik, 1997, Takeda *et al.*, 2003). The highest yield was obtained in plants with three canes, followed in decreasing order by four- and two-cane plants.

Table 3. Average yield per plant depending on cane number

	Two canes	Three canes	Four canes
Total number of fruits per plant	1492.2	2227.4	2353.0
Average fruit weight (regardless of harvest date)	6.1	6.3	5.5
Yield (kg/plant)	9.10	14.03	12.94
Yield (t/ha)	20.02	30.86	28.47

Given the 'Thornfree' cultivar specificity in that a certain percentage of fruits does not reach full maturity (Mišić and Nikolić, 2003, Šoškić, 1984), thus leading to reduced total yield, and considering the introduction of new cultivars (Milošević, 2000), the results show that, in terms of fruit yield and quality, the load of three canes per plant can be considered optimal for cost-effective production.

Conclusion

The study on the effect of a density of 2, 3 and 4 canes per plant on the yield potential of blackberry (*Rubus fruticosus* L.) cv. 'Thornfree' shows that cane density affects the number of lateral branches per cane, number of inflorescences per cane, and fruit characteristics. Reducing the number of primocanes per plant results in increased lateral branching. Inflorescences developing directly on the cane have more fruits on average than those formed on lateral branches, and they provide the highest fruiting potential in 'Thornfree'. Fruit weight is lower in plants with a higher number of canes. Total yield is the highest in plants with three canes, which is in agreement with the literature data.

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